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Upregulation of Anti-Desmocollin 3 Antibodies in Pemphigus Diseases: A Case-control Study

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ABSTRACT

Background: Pemphigus diseases are a subgroup of autoimmune bullous diseases characterized by autoantibodies against desmogleins and occasionally desmocollins. Desmocollin 3 is the main desmocollin isoform that contributes to cell adhesion in the epidermis.

Objective: To evaluate the presence and level of anti-desmocollin 3 antibodies in pemphigus diseases, and to investigate whether their presence is associated with a specific type, presentation, or clinical pattern.

Methods: Forty patients with pemphigus diseases and forty healthy controls were enrolled. Medical history, clinical examination, and pemphigus disease area index (PDAI) scoring were recorded for all patients. Serum samples were collected from both groups for assessment of anti-desmocollin 3 antibody reactivity by ELISA.

Results: The presence of anti-desmocollin 3 antibodies was significant among patients with pemphigus compared with controls (P=0.003). The level of anti-desmocollin 3 antibodies was also significantly higher in patients with pemphigus compared with controls (P=0.01). There was no significant relationship between the presence of anti-desmocollin 3 antibodies and any of the clinical presentations of pemphigus (type, severity, duration, activity, presence of annular pattern, or site of affection – mucosal, cutaneous, on the scalp, palmoplantar, or flexural).

Conclusion: Anti-desmocollin 3 antibodies are upregulated in pemphigus diseases and can contribute to the pathogenesis of pemphigus. No specific clinical type, presentation, or pattern was found to be associated with the presence of anti-desmocollin 3 antibodies.

KEY WORDS: cadherins, intraepidermal, atypical pemphigus, pemphigus vulgaris, superficial pemphigus

INTRODUCTION

Pemphigus diseases refer to a subgroup of autoimmune bullous diseases distinguished by the formation of intraepidermal blisters affecting the skin and/or mucous membranes. They can be classified into classical and non-classical pemphigus. Classical pemphigus includes pemphigus vulgaris (PV) and its subtype pemphigus vegetans, as well as pemphigus foliaceus (PF) and its subtype pemphigus erythematosus. The non-classical pemphigus group includes other rare types, such as pemphigus herpetiformis, paraneoplastic pemphigus (PNP), drug-induced pemphigus, and IgA pemphigus. The main autoantibodies identified for the pathogenesis of pemphigus diseases are anti-desmoglein 3 (Dsg3) and anti-desmoglein 1 (Dsg1) (1).

Desmocollins (Dscs) represent non-desmoglein pemphigus antigens, belonging to the desmosomal cadherins. They form, together with desmogleins, the attachment core of the desmosomes. They include Dsc1, Dsc2, and Dsc3. Each subtype is produced by a different gene (2). Desmocollin 3 (Dsc3) is the major isoform present in the desmosomes within the keratinocytes. It is mainly found in the lower layers of the epidermis (basal and first suprabasal layers) and in the outer root sheath of hair follicles (3).

This study aimed to evaluate the presence and the level of anti-desmocollin 3 antibodies in pemphigus diseases and to investigate whether their presence was associated with a specific type, presentation, or clinical pattern.

PATIENTS AND METHODS

This study was conducted at the Dermatology Outpatient Clinic, Kasr Al-Ainy Faculty of Medicine, Cairo University, after approval by the Dermatology Research Ethical Committee (DermaREC), and in accordance with the 1964 Helsinki declaration and its later amendments. Forty patients with pemphigus diseases and forty age and sex-matched healthy controls were recruited.

Patients presenting to the clinic with pemphigus diseases were enrolled, including patients with pemphigus vulgaris, superficial pemphigus (pemphigus foliaceous and pemphigus erythematosus), and atypical forms of pemphigus (pemphigus vegetans, pemphigus herpetiformis, paraneoplastic pemphigus, and IgA pemphigus). The diagnosis was confirmed by histopathological examination and immunofluorescence. Patients were older than 18 years. Both sexes were included. Patients presenting with other autoimmune bullous diseases e.g. bullous pemphigoid,

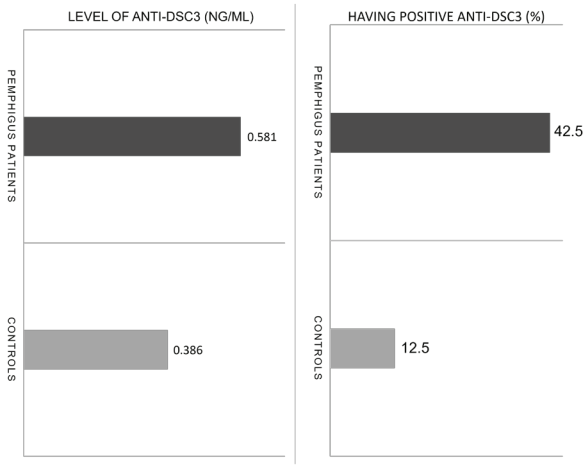

Figure 1. Comparison of the presence of anti-Dsc3 antibodies and the mean level of anti-Dsc3 antibodies between patients with pemphigus and controls.

Table 1. Demographic and clinical data of patients (n=40)

Variable		Result
Age (years)	Range	20-70
	Mean ± SD	48.3±12.9
	Median	48
Sex N (%)	Males	5 (12.5%)
	Females	35 (87.5%)
Diagnosis N (%)	Pemphigus vulgaris	21 (52.5%)
	Superficial pemphigus	13 (32.5%)
	Pemphigus foliaceus	11 (27.5%)
	Pemphigus erythematosus	2 (5.0%)
	Atypical pemphigus	6 (15%)
	Pemphigus vegetans	2 (5.0%)
	Pemphigus herpetiformis	1 (2.5%)
	IgA pemphigus	3 (7.5%)
PDAI score	Range	6-107
	Mean ± SD	29.9±20.35
	Median	23
Duration (months)	Range	3-240
	Mean ± SD	36±43.3
	Median	21
Activity N (%)	Controlled	27 (67.5%)
	In activity	13 (32.5%)

N: Number; SD: standard deviation; PDAI: Pemphigus Disease Area Index

Table 2. Comparison between patients with pemphigus and controls

	Pemphigus patients (n=40)	Controls (n=40)	*P*
Age (years) (Mean ± SD)	48.3±12.9	43.5±10.1	0.065
Gender N (%)			
- Men	5 (12.5%)	10 (25%)	0.152
- Women	35 (87.5%)	30 (75%)	
Presence of anti-Dsc3 antibodies N (%)			
Positive	17 (42.5%)	5 (12.5%)	**0.003***
Negative	23 (57.5%)	35 (87.5%)	
Anti-Dsc3 antibody mean level (ng/ml)			
Range	0.3 to 1	0.3 to 0.5	**0.010***
(Mean ± SD)	0.581±0.23	0.386±0.09	

**P* value was considered significant if <0.05; SD= standard deviation; N: Number

epidermolysis bullosa acquisita, etc. were excluded. Pregnant and lactating females were also excluded. Healthy controls enrolled were above 18 years old, with no history of other chronic dermatological or systemic disease, and were age and sex-matched to the patient group. Informed written consents were signed by patients and controls before participation.

A detailed medical history was obtained from all patients. Patients were clinically examined to determine the sites of affection (cutaneous, mucosal, mucocutaneous, scalp, palmoplantar, and flexural) and pattern of skin affection (presence or absence of annular pattern). Pemphigus disease area index (PDAI) scoring for severity of pemphigus was calculated for all patients. PDAI values from 0 to 8 were classified as follows: mild disease; 9 to 24: moderate disease; and 25 or higher: severe disease (4).

Sera were collected from all patients and controls. A five milliliter blood sample was withdrawn and centrifuged, and serum was then extracted and kept at -30 °C in a fridge. Sera were used for detection of anti-Dsc3 antibody titers using commercial Enzyme-linked Immunosorbent Assay (ELISA) kits (MyBioSource, Catalogue No. MBS7606335, USA).

Statistical analysis

Comparison of numerical variables between the two studied groups was performed using the Mann Whitney U-test for independent samples and the Chi-square (X^2) test for categorical data. The exact test was used when the expected frequency was less than 5. Correlations were calculated using the Spearman rank correlation equation. Two-sided *P* values lower than 0.05 were considered statistically significant.

RESULTS

Clinical data of the studied groups

The patient data are summarized in Table 1. There were no statistically significant differences between patients and controls with regard to age and sex (Table 2).

Comparison of anti-Desmocollin 3 antibodies between patients and controls

The presence of anti-Dsc3 antibodies was statistically significantly higher in patients with pemphigus compared with controls. Anti-Dsc3 antibody levels were also significantly higher in patients compared with controls (Table 2) (Figure 1).

Comparisons between patients with positive and negative anti-Desmocollin 3 antibodies

There were no statistically significant differences between patients with positive and negative anti-Dsc3 antibodies regarding age, duration of disease, PDAI score, severity of the disease, type of pemphigus disease, activity, site of affection, or pattern of disease (presence or absence of annular pattern) (Table 3).

DISCUSSION

Distinguishing the antigens targeted in pemphigus diseases helps to understand the pathogenesis and determine the diagnostic tools and therapeutic lines for these diseases. The role of Anti-Dsc3 antibodies in pemphigus diseases is controversial (5-7). Their

Table 3. Comparison between patients with positive and negative anti-desmocollin 3 antibodies

Variable	Anti-desmocollin 3 antibodies		*P*
	Positive (n=17)	Negative (n=23)	
Mean age (years) (mean ± SD)	46.3±12	45.8±11.8	0.691
Mean duration (months)			
(mean ± SD)	22.06±26.24	46.30±50.81	0.601
Median	12	36	
Mean PDAI score	33.9±18.7	26.9±21.4	0.139
Disease severity N (%)			
Mild	2 (11.8)	1 (4.3)	
Moderate	5 (29.4)	15 (65.2)	0.079
Severe	10 (58.8)	7 (30.4)	
Type of pemphigus disease N (%)			
Pemphigus vulgaris	7 (41.2)	14 (60.87)	
Superficial pemphigus	8 (47.06)	5 (21.7)	
Pemphigus foliaceus	6 (35.3)	5 (21.7)	
Pemphigus erythematosus	2 (11.8)	0 (0)	
Atypical pemphigus	2 (11.76)	4 (17.39)	
Pemphigus vegetans	0 (0)	2 (8.7)	0.24
Pemphigus herpetiformis	1 (5.9)	0 (0)	
IgA pemphigus	1 (5.9)	2 (8.7)	
Mucocutaneous affection N (%)			
Cutaneous	10 (58.8)	6 (26.1)	
Mucous	0 (0.0)	1 (4.3)	0.094
Mucocutaneous	7 (41.2)	16 (69.6)	
Scalp affection N (%)			
Yes	8 (47.1)	6 (26.1)	0.169
No	9 (52.9)	17 (73.9)	
Flexural Affection N (%)			
Yes	3 (17.65)	10 (43.48)	0.085
No	14 (82.35)	13 (56.52)	
Palmoplantar affection N (%)			
Yes	1 (5.88)	0 (0.00)	0.425
No	16 (94.12)	23 (100)	
Annular Pattern N (%)			
Yes	2 (11.8)	2 (8.7)	0.749
No	15(88.32)	21(91.3)	
Activity N (%)			
Controlled	10 (58.8)	17 (73.9)	0.314
In activity	7 (41.2)	6 (26.1)	

**P* value was considered significant if <0.05; SD= standard deviation; N: Number; PDAI: Pemphigus Disease Area Index

presence was initially detected in case reports of pemphigus vulgaris (6) and atypical pemphigus (8,9).

In this study, the presence of anti-Dsc3 antibodies was significantly higher among patients with pemphigus compared with controls. The level of anti-Dsc3 antibodies was also significantly higher in patients with pemphigus. This suggests a pathogenic relevance of Dsc3 autoantibodies in pemphigus diseases. These findings are consistent with the previous observation that antibodies against Dsc3 interfere with keratinocytes adhesion and induce intra-epidermal blistering. Recombinant Dsc3 can specifically protect against this pathogenic effect (10).

In the present study, there was no significant relationship between the type of pemphigus disease (pemphigus vulgaris, superficial pemphigus, or atypical pemphigus) and the presence of anti-Dsc3 antibodies. Previous case reports reported the presence of anti-Dsc3 antibodies in cases of atypical pemphigus, e.g. pemphigus herpetiformis (8,11-15),

pemphigus vegetans (9,11,16,17), and paraneoplastic pemphigus (18). In a mouse model, it was suggested that anti-Dsc3 antibodies were specifically associated with atypical presentation of pemphigus (19). However, in the current study, the presence of anti-Dsc 3 antibodies in these disease types was not statistically significant compared with patients with classic pemphigus vulgaris. This indicates that anti-Dsc3 antibodies have a role in blistering in all pemphigus diseases, regardless of the subtype.

Previously, Kamiya *et al.* (6) reported one case of pemphigus vulgaris with both IgG autoantibodies against Dsg3 and Dsc3. However, Müller *et al.* (5) and Meltzanidou *et al.* (7) did not detect anti-Dsc3 antibodies in the sera of 74 European patients and 45 Greek patients with PV, respectively. This can be explained by the fact that these studies enrolled patients of different ethnic groups. This may contribute to thoroughly clarifying whether a possible causative role of Dsc3 in pemphigus can be attributed to genetic or environmental factors.

Iranzo *et al.* (17) reported a case of pemphigus vegetans with exclusive antibodies against Dsc 1-3, while antibodies against desmogleins were absent. Histopathology of Dsc-positive pemphigus vegetans cases showed neutrophilic and eosinophilic abscesses in the epidermis with eosinophilic infiltration of the epidermis and upper-to-mid dermis (16,17).

IgA pemphigus is a less common, atypical subtype of pemphigus characterized mainly by the presence of IgA autoantibodies. Against our expectations, one of the three patients with IgA pemphigus in the present study had positive IgG anti-Dsc3 antibodies. Mixed IgG and IgA classes of autoantibodies, including desmocollins, were previously reported in IgA pemphigus, explaining the diversity in the clinical and histopathological picture of the disease (20).

In the present study, the presence of anti-Dsc3 antibodies had no significant correlation with the age of the patients and the disease duration. This suggests that the role of anti-Dsc3 antibodies in pemphigus diseases is independent of these parameters. Similarly, previous studies showed no relation between clinical data and other pemphigus autoantibodies. The presence of anti-Dsc3 antibodies also did not correlate with disease severity (PDAI score) or activity. Notably, anti-Dsg3, the main autoantibodies in pemphigus, can remain elevated during remission of the disease (21).

A clinical study on the sera of 38 patients with PV showed that patients with Dsc3, Dsg3, and Dsg1 autoantibodies had mucocutaneous disease, while patients with Dsc3 autoantibodies alone demonstrated mucosal but not skin disease (10). However, Makino *et al.* detected autoantibodies to Dsg3, Dsc2, and Dsc3 in a case of mucosal dominant-type pemphigus vulgaris with severe affection of the pharynx (22). Our study revealed no significant difference between the site of affection (mucocutaneous, cutaneous only, mucosal only) and the presence of anti-Dsc3 antibodies. It seems that mucosal involvement is related to Dsg3 rather than Dsc3.

The presence of lesion on the scalp and palmoplantar, flexural, and annular lesions also showed no significant relationship with the presence of anti-Dsc3 antibodies in the present study. One patient with pemphigus vulgaris presented with palmar blisters without keratoderma and was positive for anti-Dsc3 antibodies. Interestingly, Bolling *et al.* (23) hypothesized a pivotal role for anti-Dsc3 antibodies in the pathogenesis of acquired palmoplantar keratoderma in a case of immunobullous disease associated with an impressive acquired palmoplantar keratoderma.

The present study work highlights the possible pathogenic role of anti-Dsc3 antibodies in pemphigus diseases. However, the presence of anti-Dsc 3 antibodies can represent an epitope spreading phenomenon. Under this assumption, primary anti-desmoglein autoantibodies disrupt the desmosomes, inducing an autoimmune reaction against other desmosomal structures e.g. Dsc3. Still, autoantibodies against the secondary epitope can contribute to the progression of the disease (24). Although the pathogenic role of anti-Dsc antibodies is not yet completely understood, authors have suggested the term "anti-Dsc pemphigus" to define cases with positive IgG or IgA desmocollin antibodies (1).

In conclusion, our findings suggest a possible pathogenic role of anti-Dsc3 antibodies in pemphigus diseases. This provides further evidence for the role of Dsc3 in cell adhesion. This pathogenic role is most likely not related to a specific clinical type, pattern, or presentation of pemphigus diseases. Further studies on different ethnic groups can elucidate the exact significance of anti-Dsc 3 antibodies in pemphigus diseases.

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