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Evaluation of the possible association between acantholysis and anti-desmogleins 1 and 3 values in pemphigus vulgaris and pemphigus foliaceus

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Abstract

Objectives: Acantholysis is the main pathologic finding in pemphigus, and its location has been historically used to distinguish the major subtypes of pemphigus vulgaris (PV) and pemphigus foliaceus (PF). While suprabasal clefts are present in PV, PF includes intragranular or subcorneal clefts. After the introduction of anti-desmoglein ELISA, PF is characterized by anti-Dsg 1 and PV by anti-Dsg 3 autoantibodies. However, pathological and serological findings are not consistent in all patients. In this study, we tried to investigate the agreement between serological and pathological findings for the diagnosis of pemphigus.

Methods: We restudied the acantholysis location in skin biopsy samples of 168 pemphigus patients and compared the subtypes of pemphigus based on anti-Dsg1/3 ELISA and acantholysis locations.

Results: In 33 (19.6%), 100 (59.5%), and 35 (20.8%) of patients, acantholysis was observed in the upper half, the lower half, and throughout the epidermis, respectively. The mean anti-Dsg1 and anti-Dsg3 values were 169.76 and 43.45 U/mL in upper clefts and 120.53 and 157.88 U/mL in lower clefts, respectively. By assuming anti-Dsg1/3 as the gold standard of diagnosis of pemphigus, the sensitivity and specificity of cleft location-based diagnosis were calculated as 90.2% and 80% for PV and 80% and 90.2% for PF, respectively. There was an overall agreement of 87.97% between histological and serological diagnosis.

Conclusions: The histological findings in PV and PF are not necessarily correlated with sera antibodies' profile. Clinical manifestations, histopathological findings, direct immunofluorescence, and serologic study are all required to accurate diagnosis of the pemphigus and differentiate its subtypes.

KEYWORDS
acantholysis, anti-desmoglein antibody, pemphigus, pemphigus foliaceus, pemphigus vulgaris

1 | INTRODUCTION

Pemphigus is a group of rare autoimmune bullous diseases, characterized by blisters on skin and/or mucous membranes. It is caused by the autoantibodies against desmosomal glycoprotein expressed on keratinocytes, such as desmoglein (Dsg) 1 and Dsg 3, which result in intra-epidermal acantholysis and bullous formation.[1,2] Diagnosis of pemphigus is based on the clinical presentations, enzyme-linked immunosorbent assay (ELISA) study on Dsg 1 and 3 antibody levels, the presence of intraepithelial clefts and acantholysis findings in the pathological study, and intercellular IgG deposits on direct immunofluorescence (DIF) studies.[3–6] The two most common subtypes of pemphigus are pemphigus vulgaris (PV) and pemphigus foliaceus (PF), differentiated by the level of cleft, location of acantholysis, and anti-Dsg 1 and 3 values. Before the introduction of Dsg1/3 ELISA, PV and PF had been differentiated histologically. As a rule, in PV, acantholysis mostly occurs in the suprabasal layers, whereas in PF acantholysis predominantly occurs in the granular layer. Nowadays, in order to differentiate PV and PF, Dsg1/3 ELISA could help, since PV is characterized by the presence of anti-Dsg 3 antibodies regardless of anti-Dsg 1 antibody status, while in PF, only Dsg 1 antibodies are present.[7] Recent studies have shown that Dsg1/3 ELISA and histological findings may be discordant in some cases.[8] The aim of the present study is to investigate the association of the location of acantholysis and the level of anti-Dsg 1 and anti-Dsg 3 index value in PV and PF. We also determined the overall agreement between the histological and serological findings in the diagnosis of PV and PF to obtain a better understanding of the role of different diagnostic methods in pemphigus.

2 | METHODS

2.1 | Patients

In this study, the records of PV and PF patients who visited Razi dermatology hospital at Tehran University of Medical Sciences, Tehran, Iran between 2013 and 2016 were evaluated. The diagnosis was made based on the clinical presentations (bullae and erosions), histopathological findings (cleft level and acantholysis location), intercellular IgG and/or C3 deposition in DIF, and also a positive value for anti-Dsg 1 or 3 ELISA. This study was approved by the Ethical Committee of Tehran University of Medical Sciences (IR.TUMS.MEDICINE.REC.1396.3256).

2.2 | Data collection

The report of the ELISA index value of anti-Dsg 1 and anti-Dsg 3 was obtained from patients' medical records and subdivided into positive and negative groups with a cut-off level of 20 U/mL. In each case, pathological slides, prepared previously in the Razi Hospital, were reviewed by an expert dermatopathologist to reexamine the location of acantholysis including upper half (the granular layer or subcorneal area), lower half (including suprabasal area), and throughout the epidermis (both upper and lower parts). Moreover, in sections with acantholysis throughout the epidermis, the location was determined whether to be upper or lower dominant. The levels of circulating autoantibodies against the Dsg1 and Dsg3 were determined using ELISA kit (EUROIMMUN, Medizinische Labordingnostika AG) in serum samples. According to the serological evaluation, the patients with a positive index value of anti-Dsg 1 and a negative index value of anti-Dsg 3 were assigned into PF group, whereas whom with a positive index value of anti-Dsg 3 regardless of the status of their anti-Dsg 1 index value were assigned to PV group.

2.3 | Statistical analysis

SPSS version 24 was used to investigate the association of the ELISA index value of anti-Dsg 1 and 3 and the location of acantholysis in PV and PF patients (upper half, lower half, and throughout the epidermis) using chi-square test. Ten cases with negative values of anti-Dsg 1 and 3 and histological features of pemphigus were excluded from the study.

By assuming anti-Dsg1/3 ELISA as the gold standard for differentiating PV and PF, the correlation of the anti-Dsg 1 and 3 index values and the location of acantholysis were also assessed using *t* test in PV and PF patients, separately. The sensitivity and specificity of histology-based diagnosis (location of acantholysis) were also estimated. PV cases were divided into two different subgroups including acantholysis at the lower half of epidermis and the rest of the PV patients including whom with acantholysis throughout the epidermis and upper part of the epidermis. Similarly, PF cases were divided into A. upper half epidermal acantholysis and lower half and throughout the epidermal acantholysis. Since all samples of skin biopsies belonged to untreated patients, the sensitivity and the specificity of the pathologic study were not affected by treatment.

The overall agreement of histological and serological methods, regarding differentiating PV and PF cases, was calculated. *P*-value <.05 was considered statistically significant.

3 | RESULTS

3.1 | Patients characteristics

In total, 168 patients, aging from 20 to 84 years old (mean of 49 ± 14.2) consisted of 94 men (56%) and 74 women (44%), were included. There were 123 cases of PV and 35 cases of PF, distinguished based on the status of anti-Dsg 1 and anti-Dsg 3 ELISA. In 10 patients,

the serological test was negative for both anti-Dsg 1 and 3. Although their pathological characteristics were consistent with PV in eight and with PF in two, they were assigned in neither PV nor PF groups. Anti-Dsg 1 and 3 values ranged from 0.7 to >200 U/mL (mean: 138.74 U/mL) and 1 to >200 U/mL (mean: 126.61 U/mL), respectively.

3.2 | Histopathological findings

Histopathological findings revealed that the location of acantholysis was on the upper half of the epidermis in 33 (19.6%) cases, the lower half of epidermis in 100 cases (59.5%), and throughout the epidermis in 35 (20.8%) cases. Figure 1 shows pathological picture of upper, lower, and throughout areas. In the PV group, acantholysis was in the upper half of epidermis in eight cases (6.5%), in the lower half in 90 cases (73.17%), and throughout the epidermis in 25 cases (20.33%) (21 cases were lower dominant, and four cases were upper dominant). Patients with PF had acantholysis in the upper half of epidermis in 23 cases (65.71%), the lower half of epidermis in four cases (11.43%), and throughout the epidermis in eight cases (22.86%) (five cases were upper dominant, and three were lower dominant; Table 1).

3.3 | The association between anti-Dsg antibodies and histopathological findings

As Table 1 presents, the serology for anti-Dsg 1 was positive in 31 out of 33 cases with upper half epidermal acantholysis, 76 out of 100 cases with lower half epidermal acantholysis, and 31 out of 35 cases with acantholysis throughout the epidermis (*P*-value = .035).

Similarly, assessing the status of anti-Dsg 3 revealed that among cases with acantholysis in the upper half of epidermis, eight cases (eight out of 33 cases), in lower epidermal acantholysis 90 out of 100 cases, and in patients with acantholysis throughout the epidermis, 25 cases had positive anti-Dsg 3 (*P*-value < .001).

Additionally, the mean index values of anti-Dsg 1 and 3 were 169.76 and 76.49 U/mL in patients with acantholysis in the upper half, 120.53 and 157.88 U/mL in the lower half, and 161.56 and 115.73 U/mL in patients with acantholysis throughout their epidermis, respectively (*P*-value < .001). In PV patients, the mean level of anti-Dsg 1 value was 127.18 U/mL in lower epidermal acantholysis and 161.74 U/mL in others (upper half and throughout the epidermis, *P*-value = .025). The mean level of anti-Dsg 3 value in PV patients was 174.67 U/mL in cases with acantholysis in the lower half of epidermis and 161.91 U/mL in the rest, *P*-value = .237 (Table 2A). In the PF patients, the mean index value of anti-Dsg 1 was 187.18 U/mL in patients with acantholysis in the upper part of the epidermis, and 182.17 U/mL with acantholysis in the lower part or throughout the epidermis, *P*-value = .679. The mean index value of anti-Dsg 3 was 4.13 U/mL in the group who had acantholysis in the upper half of epidermis and 3.34 U/mL in rest of them (lower or throughout the epidermis, *P*-value = .536) (Table 2B). Figure 2 demonstrates the dot plot graph for anti-Dsg1 vs anti-Dsg3.

Based on histopathological features regardless of characteristics of their sera, 118 cases were diagnosed as PV and 40 cases as PF subgroups. Using anti-Dsg1/3 as the gold standard of diagnosis

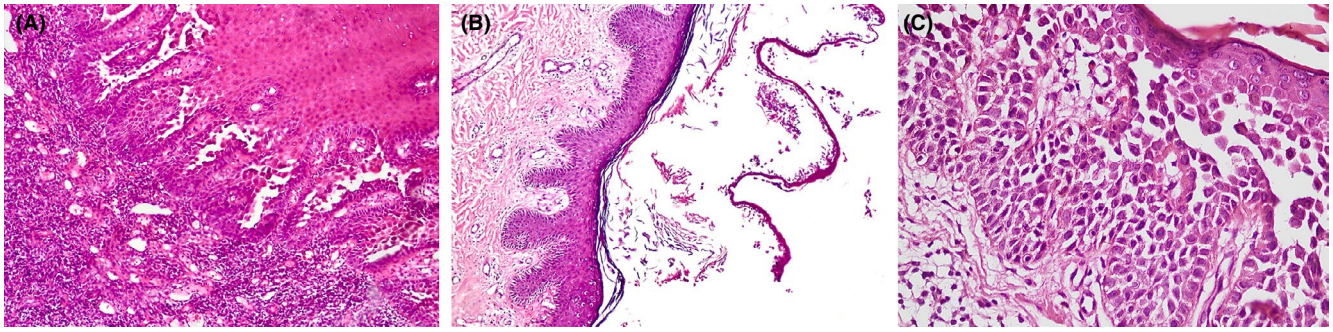

FIGURE 1 Acantholysis in the suprabasal, subcorneal, and throughout areas (A, B, and C, respectively) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 General features

Location of acantholysis	Upper half of epidermis (n = 33)	Lower half of epidermis (n = 100)	Throughout the epidermis (n = 35)	*P* value
Dsg1 index value, mean (±SD)	169.76 (±59.41)	120.53 (±81.7)	161.56 (±65.62)	.001
Dsg3 index value, mean (±SD)	76.49 (±43.45)	157.88 (±69.23)	115.73 (±88.04)	<.001
Dsg1 positive, n	31	76	31	.035
Dsg3 positive, n	8	90	25	<.001
Disease subtype				
PV	8	90	25	<.001
PF	23	4	8	

TABLE 2 (A) Comparison of features in PV cases with different acantholysis locations. (B) Comparison of features in PF cases with different acantholysis locations

Location of acantholysis	Lower half of epidermis (n = 90)	Upper half and throughout the epidermis (n = 33)	*P* value
Anti-Dsg1 index value, mean (±SD) U/mL	127.18 (±79.8)	161.74 (±58.66)	**.025**
Anti-Dsg3 index value, mean (±SD) U/mL	174.67 (±49.78)	161.91 (±60.12)	.237
Dsg1 positive, n (%)	72 (80%)	31 (94%)	**.05**
Location of acantholysis	**Upper half of epidermis (n = 23)**	**Lower half and throughout the epidermis (n = 12)**	***P* value**
Anti-Dsg1 index value, mean (±SD) U/mL	187.18 (±33.50)	182.17 (±34.03)	.679
Anti-Dsg3 index value, mean (±SD) U/mL	4.13 (±3.95)	3.34 (±2.45)	.536

Significant *P* values (<.05) are in bold.

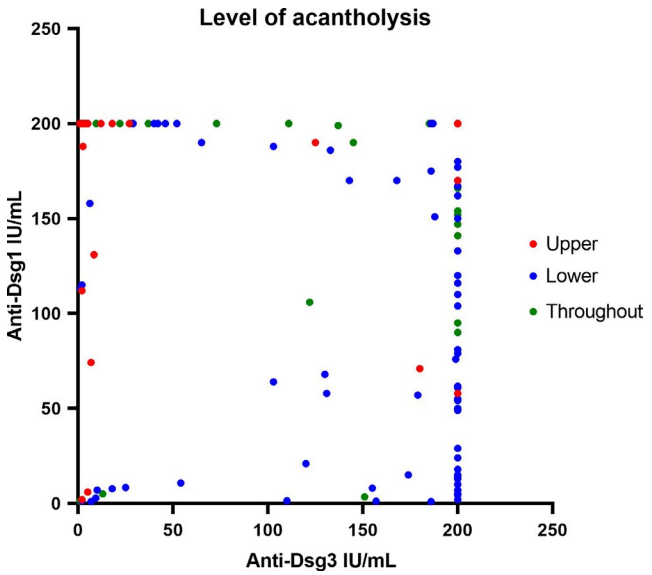

FIGURE 2 Dot plot graph for anti-Dsg1 vs anti-Dsg3. Red, blue, and green dots are representative of upper, lower, and throughout the epidermis, respectively. In this graph, values, more than 200 IU/mL, are shown as 200, due to limitations of our kit to measure higher values than 200 IU/mL [Color figure can be viewed at wileyonlinelibrary.com]

of pemphigus, the sensitivity and specificity of cleft location-based diagnosis were 90.2% and 80% for PV and 80% and 90.2% for PF, respectively. The overall agreement of anti-Dsg ELISA results and histopathology for differentiating PV and PF was 87.97%.

4 | DISCUSSION

In this study, PV patients had significantly higher anti-Dsg 3 and lower anti-Dsg 1 index values compared to the PF cases. There was a significant association between the location of acantholysis and the mean index value of anti-Dsg 1 in PV, but not in PF patients. The location of acantholysis did not correlate with the mean index value of anti-Dsg 3 in PV and PF; however, after excluding the cases that had acantholysis throughout the epidermis, the mean value of anti-Dsg 1 was significantly higher in patients with upper vs lower epidermal acantholysis.

Diagnosis of pemphigus is mainly through clinicopathological and DIF findings; however, identifying the serum values of anti-Dsg1/3 using ELISA in recent years has been suggested to enhance the sensitivity of the disease diagnosis and could be utilized to differentiate the subtypes of pemphigus.[9] The study of Mortazavi et al[10] showed that using Dsg1/3 ELISA testing in addition to pathological and DIF studies could increase the diagnostic yield in PV. However, some recent studies had reported the discrepancy between the clinicopathological and ELISA findings in diagnosis and differentiating pemphigus subtypes. For instance, investigation of Herrero-Gonzalez et al[11] on 40 patients with pemphigus revealed that the profile of antibodies and clinical findings was not concordant in 10% of cases. Similarly, Cozzani et al[12] demonstrated that distinguishing subtypes of pemphigus could not always be possible by sera antibody profiles, and they suggested that nonpathogenic anti-Dsg 3 antibodies could explain the observed dissociation. Moreover, the study of Ohata et al revealed that although the clinical and histopathological study is enough for the diagnosis of pemphigus diseases in most patients, there are still some complicated cases, such as cutaneous PV, mucocutaneous PF, or patients with acantholysis throughout the epidermis, which could not be correctly differentiated histopathologically. Their study suggests that quantifying the index value of Dsg is useful in the cases that do not exhibit the typical findings of PV and PF.[8]

The current investigation validates the results of the previous studies regarding the discordance between the pathological and serological characteristics in PV and PF. As mentioned before, the location of acantholysis in the upper epidermis was only correlated with the mean anti-Dsg 1 index value in PV. On the other hand, in 10 cases that the histopathology and DIF findings were compatible with pemphigus, serology tests were negative for both anti-Dsg 1 and anti-Dsg 3. This finding might be explained by low titer or the expression of different haplotypes of anti-Dsg or the presence of other antibodies besides anti-Dsg 1 and 3 which are involved in the pathogenesis of pemphigus.[13]

In this study, we investigated the association between the histological features and the antibodies' profile in Iranian population. As it is well-known, the serological characteristics of pemphigus

are strongly related to the genetic factors and racial differences[13]; accordingly, the profile of antibodies was determined qualitatively through measuring the mean index value of anti-Dsg 1 and 3. Besides, the considerably larger sample size of our study could confirm the findings of the previous studies with a high level of significance. Since the data were collected retrospectively, DIF study and its correlation with antibodies' profile have not been evaluated. Further investigations of pathological features such as the presence of inflammatory and acantholytic dyskeratotic cells, as well as considering the severity of disease at the time of the presentation, are highly recommended in future studies.

Taken together, the overall agreement of these two diagnostic tools for PV and PF (ie, pathological assessment of the location of acantholysis and ELISA value of anti-Dsg 1, 3) was 89.97% in the present study. Although this correlation is significant, it is not perfect and some patients may have discordant results. In these cases, it is not known which diagnostic tools should be relied on most. The array of clinical, pathological, and serological findings and following up the course of the disease may be complementary to each other.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required.

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REFERENCES

1. Stanley JR, Amagai M. Pemphigus, bullous impetigo, and the staphylococcal scalded-skin syndrome. N Engl J Med. 2006;355(17):1800–10.
2. Tsunoda K, Ota T, Saito M, Hata T, Shimizu A, Ishiko A, et al. Pathogenic relevance of IgG and IgM antibodies against desmoglein 3 in blister formation in pemphigus vulgaris. Am J Pathol. 2011;179(2):795–806.
3. Kolivras A, Gheeraert P, André J. Nail destruction in pemphigus vulgaris. Dermatology. 2003;206(4):351–2.
4. Uzun S, Durdu M. The specificity and sensitivity of Nikolskiy sign in the diagnosis of pemphigus. J Am Acad Dermatol. 2006;54(3):411–5.
5. Harman K, Gratian M, Seed P, Bhogal B, Challacombe S, Black M. 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.
6. Mihai S, Sitaru C. Immunopathology and molecular diagnosis of autoimmune bullous diseases. J Cell Mol Med. 2007;11(3):462–81.
7. Bystryn J-C, Rudolph JL. Pemphigus. Lancet. 2005;366(9479):61–73.
8. Ohata C, Ishii N, Furumura M. Locations of acantholysis in pemphigus vulgaris and pemphigus foliaceus. J Cutan Pathol. 2014;41(11):880–9.
9. 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;140:351–7.
10. Mortazavi H, Shahdi M, Amirzargar AA, Naraghi ZS, Valikhani M, Daneshpazhooh M, et al. Desmoglein ELISA in the diagnosis of pemphigus and its correlation with the severity of pemphigus vulgaris. Iran J Allergy Asthma Immunol. 2009;8(1):53–6.
11. Herrero-Gonzalez JE, Mascaró JM, Iranzo P, Herrero C. Atypical pemphigus: discordance between clinicopathological findings and the antigenic profile in four cases. J Cutan Pathol. 2006;33(7):502–7.
12. Cozzani E, Di Zenzo G, Riva S, Calabresi V, Sera F, Drosera M, et al. Are clinical phenotype and autoantibody profile always concordant in pemphigus? A study in a cohort of pemphigus patients. Eur J Dermatol. 2013;23(1):40–8.
13. 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.

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