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Original Article

Desmoglein Autoantibodies and Disease Severity in Pemphigus Patients – Correlations and Discrepancies

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Abstract

Aim: To assess the correlation between the levels of anti-desmoglein-1 and anti-desmoglein-3 autoantibodies and disease severity in pemphigus patients.

Materials and methods: Thirty-eight pemphigus patients aged 30 to 87 years were included in the study. All patients underwent clinical examination, pemphigus disease zone index assessment, histopathological and direct immunofluorescence tests, and assessment of desmoglein-1 and desmoglein-3 autoantibodies by enzyme-linked immunosorbent assay.

Results: Twenty-eight out of 38 serum samples exceeded the cut-off value of anti-desmoglein-1, and 26 of 38 sera had positive anti-desmoglein-3 antibodies. One serum from 38 controls had positive anti-desmoglein-1 antibodies. Seven (18.4%) patients experienced a mild course of the disease, 16 (42.1%) patients experienced moderate, and 15 (39.5%) patients suffered from severe pemphigus. A significant correlation between disease severity and both autoantibody levels was observed, but there were exceptions.

Conclusions: There is a significant correlation between anti-desmoglein antibodies and disease severity in the entire group, but there are also discrepancies in some cases.

Keywords

anti-desmoglein-1, anti-desmoglein-3, pemphigus disease area index, pemphigus foliaceus, pemphigus vulgaris

INTRODUCTION

Pemphigus comprises a group of rare potentially life-threatening autoimmune bullous diseases. Pemphigus vulgaris (PV) is the most common form. Mucosal lesions are present in almost all patients, with or without cutaneous bullae or erosions.[1,2] The pathogenesis of pemphigus is characterized by acantholysis and intraepidermal cavity formation resulting from immunoglobulins class G (IgG) directed against desmoglein-1 (Dsg1) and/or desmoglein-3 (Dsg3).[3-5] Dsg1 and Dsg3 are cadherins, which are calcium-dependent transmembrane glycoproteins of the epidermal or epithelial desmosomes. They are part of the maculae adherentes and allow the cell-to-cell adhesion in the epidermis and the epithelium.[6]

AIM

The aim of this study was to assess the correlation between the levels of anti-desmoglein-1 and anti-desmoglein-3 autoantibodies and disease severity in pemphigus patients.

MATERIALS AND METHODS

Patients

This prospective study was conducted from March 2020 to November 2022. Thirty-eight patients with pemphigus were included based on the following criteria: (1) clinical diagnosis, (2) histopathological picture revealing intraepidermal acantholysis, and (3) deposition of IgG and complement component 3 on keratinocyte surface established by direct immunofluorescence assay. The severity of pemphigus was assessed by the validated Pemphigus Disease Area Index (PDAI) scoring system.[7-9] A control group of 38 people (17 patients with bullous pemphigoid, two patients with psoriasis, five pregnant woman, and 14 healthy blood donors) served as controls. Controls were sex and age matched. The study was approved by the Ethics Committee of the Medical University of Plovdiv and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

Anti-desmoglein-1 and anti-desmoglein-3 enzyme-linked immunosorbent assay (ELISA)

Anti-Dsg1 and anti-Dsg3 antibodies (Abs) were evaluated using the EUROIMMUN anti-desmoglein-1 and anti-desmoglein-3 ELISA (IgG) test kit according to the manufacturer's instructions. Sera from pemphigus patients and controls were collected and stored at –80°C until assayed. The patient sera were diluted 1:101 in sample buffer. A value exceeding or equal to 20 RU/ml was deemed to be positive, and a value <20 RU/ml was interpreted as negative (EUROIMMUN Medizinische Labordiagnostika, Lübeck, Germany).

Statistical analysis

Continuous variables are given as means and standard deviations (±SD), and category variables as percentages. The Spearman correlation test was used to compare disease severity and autoantibody levels. Sensitivity and specificity of ELISA were analyzed with the McNemar test. A receiver operating characteristic (ROC) curve was calculated to determine a cutoff value for anti-Dsg1 and anti-Dsg3 Abs. Statistical analyses were performed with data analysis software IBM – SPSS v. 23. Statistical significance was considered significant at $p<0.05$.

RESULTS

The patients with pemphigus had a mean age of 56.63±15.330 years; there were 27 female patients (71.1%) and 11 male patients (28.9%). PV was diagnosed in 31 patients (81.6%) and seven patients (18.4%) were with pemphigus foliaceus (PF). The severity of pemphigus was measured using the PDAI scoring system, which ranged from 2 to 250 points. The disease was mild in seven (18.4%) patients, moderate in 16 (42.1%), and severe in 15 (39.5%) patients. Mucocutaneous phenotype was established in 26 patients (68.4%), oral pemphigus in five (13.2%), and cutaneous pemphigus in seven (18.4%) cases. Anti-Dsg1 Abs were positive in 28 (73.7%) of 38 patients, and anti-Dsg3 Abs in 24 of 31 (77.4%) patients with PV, and in two (28.6%) of the PF patients. Anti-Dsg1 Abs were found in three patients (60%) with mucosal PV. Six patients (85.7%) with PF exhibited highly positive anti-Dsg1 Abs (>200,000 RU/ml). One of the PF patients had negative anti-Dsg1 (10.957 RU/ml) and anti-Dsg3 Abs (12.99 RU/ml). One of the 38 controls, a patient with bullous pemphigoid, had positive anti-Dsg1 Abs in low level (41.307 RU/ml). The sensitivity of ELISA for pemphigus was 84.21%, at a specificity of 97.37 % and efficiency of 90.79% ($p>0.05$) (McNemar test). ROC analysis revealed high overall diagnostic performance for anti-Dsg1 Abs ($p<0.001$) and anti-Dsg3 Abs ($p<0.001$) **(Figs 1, 2)**. New cutoff values of anti-Dsg1 and anti-Dsg3 Abs calculated from the ROC curve were proposed providing a 74% sensitivity, an 82% specificity for anti-Dsg1 Abs and 84% sensitivity, a 100% specificity for anti-Dsg3 Abs **(Tables 1, 2) (Figs 1, 2)**. A significant correlation was found among 38 patients between their PDAI scores and levels of anti-Dsg1 Abs ($p<0.05$; $r=0.525$) and anti-Dsg3 Abs also ($p<0.05$; $r=0.399$), and among 26 mucocutaneous phenotype patients: PDAI and anti-Dsg1 Abs ($p<0.05$; $r=0.600$), and PDAI and anti-Dsg3 Abs ($p<0.05$; $r=0.602$) **(Figs 3, 4)**.

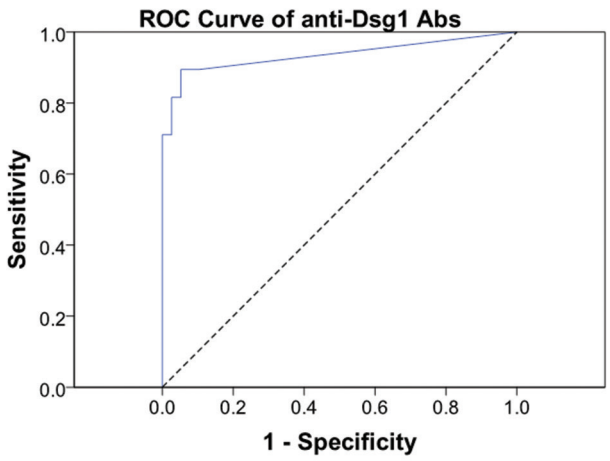

Figure 1. ROC and AUC of anti-Dsg1 Abs for determining the optimal cut-off value.

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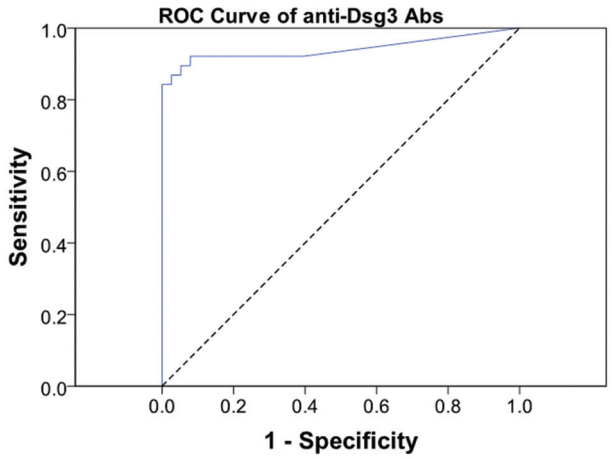

Figure 2. ROC and AUC of anti-Dsg3 Abs for determining the optimal cut-off value.

Table 1. Coordinates of the anti-Dsg1 Abs ROC

Desmoglein-1 positive if greater than or equal to	Sensitivity	1 - Specificity
7.75550	0.868	0.053
7.81900	0.842	0.053
8.97900	0.816	0.053
10.54500	0.816	0.026
13.86650	0.789	0.026
16.81350	0.763	0.026
19.30100	0.737	0.026

Table 2. Coordinates of the anti-Dsg3 Abs ROC

Desmoglein-3 positive if greater than or equal to	Sensitivity	1 - Specificity
5.44550	0.868	0.053
6.38600	0.868	0.026
7.32900	0.842	0.026
7.95950	0.842	0.000
8.81350	0.816	0.000
10.01150	0.789	0.000
10.77400	0.763	0.000

DISCUSSION

Twenty (52.6%) of the 38 pemphigus patients in the current study were newly diagnosed, 15 (39.5%) were receiving therapy, and three (7.9%) had ceased their medication on their own volition at the time of the ELISA testing.

A significant correlation between the PDAI scores and the ELISA tests results was revealed. Anti-Dsg1 Abs showed stronger correlation with the disease severity compared with

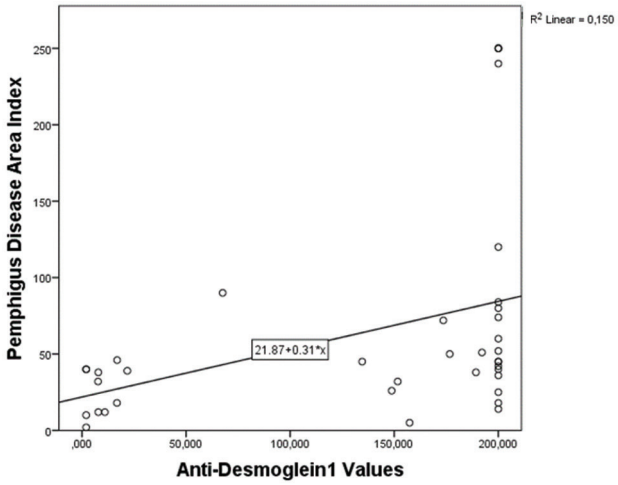

Figure 3. Significant correlation between anti-Dsg1 Abs and PDAI ($p<0.05$, $r=0.525$).

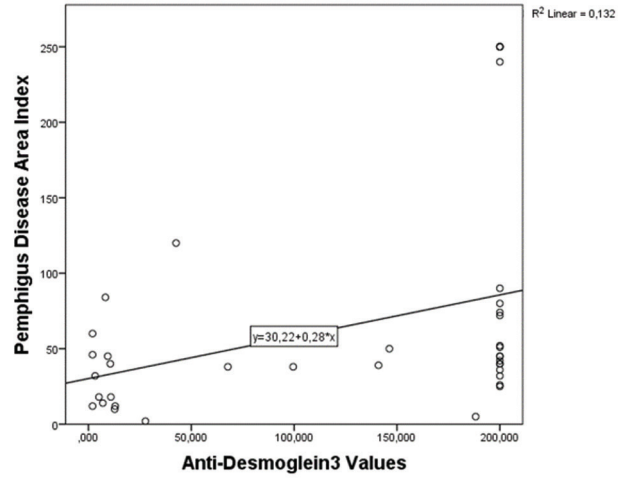

Figure 4. Significant correlation between anti-Dsg3 Abs and PDAI ($p<0.05$, $r=0.399$).

anti-Dsg3 Abs. The results of this study were consistent with other reports.[10-12] The correlation between the antibodies and PDAI in the present study was most significant in patients with mucocutaneous PV. Greek researchers concluded that patients with mucocutaneous phenotypes had a total PDAI score strongly correlated with the levels of anti-Dsg1 Abs and less strongly with the anti-Dsg3 Ab levels.[13]

In the current study, a discrepancy was seen in a patient with severe relapse of the disease with a PDAI score of 86 and negative anti-Dsg1 Abs (16.581 RU/ml) and anti-Dsg3 Abs (<2.000 RU/ml). Another incongruity occurred in a case of mild pemphigus (PDAI=13) and highly positive levels of both autoantibodies. No significant correlation was reported between anti-Dsg1 and anti-Dsg3 ELISA Abs and disease severity in some patients.[14] It was published that anti-Dsg1 ELISA tests can be considered a predictive tool for the occurrence of cutaneous relapses, whereas anti-Dsg3 Ab ELISA values do not necessarily correspond to mucosal involvement.[15] A study on 72 patients with

PV established that a single blood sample was enough to detect a significant correlation between disease severity and levels of both anti-Dsg1 and anti-Dsg3 Abs (p<0.001, p=0.020).[16] Chinese authors demonstrated that there was no correlation between conventional anti-Dsg3 Ab ELISA values and the PDAI score, whereas a positive correlation between conformational anti-Dsg3 ELISA values and activities of cutaneous lesions was found in 29 PV patients.[17] Researchers clarified that anti-desmoglein Abs significantly increased with worsening of the disease, whereas indirect immunofluorescence titers did not show correlation with disease activity.[18] According to Cheng et al., only appropriate dilutions in ELISA, such as 1:800 and 1:1600 or above, can provide true serological information about disease activity.[19]

This research established a case of newly diagnosed mucosal PV with negative anti-Dsg3 Ab (10.911 RU/ml) and anti-Dsg1 Ab levels (16.776 RU/ml). Balighi et al. also found initial negative anti-Dsg1/anti-Dsg3 Ab levels in eight female pemphigus patients.[20]

An explanation for these contradictory ELISA results could be the pathogenic non-desmoglein Abs, such as desmocollins, plakophilin3, thyroid peroxide antibodies, anti-mitochondrial proteins, cholinergic receptors, e-cadherin, and plakoglobin.[21] Negative levels of anti-Dsg1 and anti-Dsg3 Abs were reported in one of 38 pemphigus cases.[22] In this study, one pregnant female patient with pemphigus herpetiformis had high levels of anti-Dsg1 and anti-Dsg3 Abs (>200.000 RU/ml). Antibodies from patients with PF are targeted primarily against Dsg1.[23] It was established that exfoliative toxin A, which causes bullous impetigo and staphylococcal scalded skin syndrome, cleaves Dsg1, but does not target Dsg3.[24,25] The incongruity between autoantibody profile and clinical phenotype in PF patients could be due to non-pathogenic anti-Dsg3 Abs.[14]

The results of our study support previous reports on sensitivity and specificity of anti-Dsg1 and anti-Dsg3 ELISA tests for the diagnosis of pemphigus.[26-28] In particular, autoantibody levels should not be interpreted regardless of the clinical findings.

The study's limitation is the insufficient number of cases included in it.

CONCLUSIONS

Anti-desmoglein-1 and anti-desmoglein-3 antibody levels measured by ELISA can be associated with the disease activity and severity in patients with pemphigus, but there are exceptions.

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Competing interests

The authors have declared that no competing interests exist.

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Аутоантитела к десмоглеину и тяжесть заболевания у пациентов с пузырчаткой – корреляции и расхождения

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Резюме

Цель: Оценить корреляцию между уровнями аутоантител к десмоглеину-1 и антидесмоглеину-3 и тяжестью заболевания у пациентов с пузырчаткой.

Материалы и методы: В исследование включены 38 больных пузырчаткой в возрасте от 30 до 87 лет. Всем пациентам проведено клиническое обследование, оценка индекса зоны заболевания пузырчаткой, гистопатологические и прямые иммунофлуоресцентные исследования, а также определение аутоантител к десмоглеину-1 и десмоглеину-3 методом иммуноферментного анализа.

Результаты: В двадцати восьми из 38 образцов сыворотки содержание анти-десмоглеина-1 превышало пороговое значение, а в 26 из 38 сывороток были обнаружены положительные антитела к десмоглеину-3. Одна сыворотка из 38 контрольных групп содержала положительные антитела к десмоглеину-1. У семи (18.4%) больных установлено лёгкое течение заболевания, у 16 (42.1%) – среднетяжёлое, у 15 (39.5%) – тяжёлое течение заболевания. Наблюдалась значительная корреляция между тяжестью заболевания и уровнями обоих аутоантител, но были и исключения.

Заключение: Существует значительная корреляция между антителами к десмоглеину и тяжестью заболевания во всей группе, но в некоторых случаях наблюдаются и расхождения.

Ключевые слова

антидесмоглеин-1, антидесмоглеин-3, индекс площади заболевания пузырчаткой, pemphigus foliaceus, pemphigus vulgaris