

Using Desmoglein 1 and 3 Enzyme-linked Immunosorbent Assay as an Adjunct Diagnostic Tool for Pemphigus

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Background: Pemphigus is an acquired autoimmune intraepidermal blistering disease that is divided into 2 major subtypes: pemphigus vulgaris (PV) and pemphigus foliaceus (PF). Patients with pemphigus have circulating anti-desmoglein (Dsg)1 and/or anti-Dsg3 IgG autoantibodies. Recently, a novel commercial enzyme-linked immunosorbent assay (ELISA) against Dsg1 and Dsg3 has been established and found to be extremely sensitive and specific. To date, the usefulness of Dsg1 and Dsg3 ELISA in the diagnosis of pemphigus in the Taiwanese population has never been reported.

Methods: Serum samples were obtained from 143 patients, including 20 patients with PV, 9 patients with PF, 72 patients with bullous pemphigoid, 1 patient with dermatitis herpetiformis and 41 patients with non-autoimmune blistering diseases. They were tested for anti-Dsg1 and anti-Dsg3 reactivity by ELISA.

Results: Seventeen of 20 PV sera (85%) exceeded the cut-off value of Dsg3 ELISA, and 9 of 9 PF sera (100%) exceeded the cut-off value of Dsg1 ELISA, while only 1 (0.88%) and 3 (2.6%) of 114 non-pemphigus sera exceeded the cut-off values of Dsg3 and Dsg1 ELISAs, respectively. Thus, the sensitivity and specificity of Dsg3 ELISA were 85% and 99.1%, while the sensitivity and specificity of Dsg1 ELISA were 100% and 97.4%, respectively. The correlation between ELISA scores and disease activity along the time course was examined in 6 PV patients and 1 PF patient, and the result was equivocal.

Conclusion: Dsg1 and Dsg3 ELISAs provide a simple, highly sensitive and specific method that can serve as a useful adjunct tool for the initial diagnosis of pemphigus. [*J Chin Med Assoc* 2007;70(2):65–70]

Key Words: desmoglein 1, desmoglein 3, enzyme-linked immunosorbent assay, pemphigus foliaceus, pemphigus vulgaris

Introduction

Pemphigus is a group of acquired autoimmune blistering diseases of the skin and/or mucous membranes characterized by autoantibodies against the cell surface of epidermal keratinocytes.¹ There are 2 major subtypes in our daily practice: pemphigus foliaceus (PF) and pemphigus vulgaris (PV). The 2 types show distinct clinical and histopathologic features and are usually distinguishable from each other. PF patients display clinically superficial blisters and erosions mainly on the face and trunk without involvement of the oral mucosa, and histopathologically show subcorneal acantholytic bullae. In contrast, PV patients present clinically with

flaccid blisters and persistent erosions preferentially on the intertriginous regions, almost always associated with oral mucosal lesion, and histopathologically show suprabasilar acantholytic bullae in the lower epidermis. Recent progress in molecular biology has revealed that the major antigens in PF and PV are the desmosomal glycoproteins desmoglein 1 (Dsg1) and desmoglein 3 (Dsg3),^{2–6} respectively. Both Dsg1 and Dsg3 are extracellular domains of the desmosomal adhesion molecules that belong to the cadherin family, are expressed in stratified squamous epithelia and play pathogenic roles in blister formation in PF and PV, respectively. Up till now, the diagnosis of autoimmune blistering diseases has relied on clinical manifestations, histopathologic

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findings and either direct immunofluorescence (DIF) or indirect immunofluorescence (IIF). However, the result of immunofluorescence may be negative in some cases, and the cell staining pattern of IF test is identical between PV and PF. Recently, a novel enzyme-linked immunosorbent assay (ELISA) against Dsg1 and Dsg3 has become commercially available and found to be extremely sensitive and specific.⁷⁻¹² Moreover, recent studies show that ELISA index values fluctuate in parallel with disease activity, and provides a valuable tool to monitor disease activity.^{7,8,13-15}

To date, the usefulness of Dsg1 and Dsg3 ELISAs in the diagnosis and monitoring of the disease activity of pemphigus in the Taiwanese population has never been reported. Given these considerations, we collected sera from pemphigus patients, and performed Dsg1 and Dsg3 ELISAs to determine the sensitivity and specificity of the ELISA test. We also checked if the ELISA scores correlated with clinical disease activity.

Methods

Human sera

From February 2003 to April 2006, subjects with clinically blistering diseases were included in our study. We performed skin biopsies and collected serum from these subjects before treatment. Histopathologic examinations including hematoxylin-eosin stain, DIF (IgG, IgA, IgM, C3 and type IV collagen included) and IIF were performed. All serum samples were also tested with Dsg3 and Dsg1 ELISAs. Serial serum samples from 6 patients with PV and 1 patient with PF were collected and used for correlation with disease activity.

Dsg3 and Dsg1 ELISAs

Dsg3 and Dsg1 were measured using ELISA (Medical and Biological Laboratories Co. Ltd., Nagoya, Japan). The intraassay and interassay variations were both < 15% for each sample according to the manufacturer's instructions. This assay is generally not interfered with by factors such as the level of hemoglobin, bilirubin, or rheumatoid factor, but using highly hemolysed samples or highly lipemic samples should be avoided.

Statistical analysis

All statistical data are presented as mean \pm standard error of the mean (SEM). We used the generalized linear model repeated measurement method to determine the correlation between the ELISA scores and disease activity. Comparison between groups was calculated using Student's *t* test.

Table 1. Anti-desmoglein (Dsg)1 and anti-Dsg3 value (unit/mL) of each pemphigus vulgaris (PV) and pemphigus foliaceus (PF) patient*

	PV		PF		
	Dsg3	Dsg1	Dsg3	Dsg1	
PV 1	150.7	44.4	PF 1	0	184.0
PV 2	76.3	141.27	PF 2	3.7	52.3
PV 3	235.3	216.6	PF 3	0.8	185.0
PV 4	126.81	223.75	PF 4	9.9	226.4
PV 5	193.48	255.0	PF 5	0.7	173.2
PV 6	193.23	245.09	PF 6	0	193.8
PV 7	198.7	164.4	PF 7	0.6	191.9
PV 8	158.2	67.1	PF 8	1.0	200.2
PV 9	163.9	106.7	PF 9	1.1	131.1
PV 10	150.8	98.0			
PV 11	158.0	164.0			
PV 12	203.6	174.7			
PV 13	151.7	113.6			
PV 14	67.3	72.6			
PV 15	27.9	99.6			
PV 16	27.7	109.2			
PV 17	37.6	30.7			
PV 18	15.5	90.6			
PV 19	0.6	4.6			
PV 20	0.1	176.6			

*Cut-off positive index value of anti-Dsg1 and anti-Dsg3 was 20 unit/mL.

Results

Characteristics of patients with pemphigus

Sera were obtained from 143 patients with clinically blistering disease, including PF ($n=9$), PV ($n=20$), bullous pemphigoid (BP) ($n=72$), dermatitis herpetiformis (DH) ($n=1$) and non-autoimmune mediated blistering diseases ($n=41$). Diagnoses were confirmed via histopathologic and immunofluorescence tests, and all patients from both PV and PF groups showed positive DIF with IgG or C3 staining of keratinocyte cell surface. The average ages of PV patients and PF patients were 56 ± 3.38 years (range, 26-77 years) and 68.3 ± 6.94 years (range, 29-91 years), respectively (Table 1). The male: female ratios in PV and PF patients were 7:3 (14 males, 6 females) and 2:1 (6 males, 3 females), respectively.

Dsg1 and Dsg3 ELISA analysis from pemphigus patients and controls

For Dsg1 ELISA, 9 of 9 sera from PF patients (100%) and 19 sera from 20 PV patients (95%) exceeded the cut-off value, while 3 of 72 sera from BP patients (4.2%), none from the DH patient, and none of 41 sera from patients with non-autoimmune bullous diseases (0%) exceeded the cut-off value (Table 2). For Dsg3

Table 2. Demographic data of pemphigus vulgaris (PV) and pemphigus foliaceus (PF) patients

	PV (n=20)	PF (n=9)
Age, yr*	56 ± 3.38	68.3 ± 6.94
Male/female, n	14/6	6/3
	Dsg3	Dsg1
Sensitivity (%)	85.0	100.0
Specificity (%)	99.1	97.4
Positive predictive value	0.94	0.75
Negative predictive value	0.97	1.00
Positive likelihood ratio	96.9	38.0
Negative likelihood ratio	0.151	0

*Mean ± standard error of the mean.

ELISA, 17 of 20 sera from PV patients (85%) showed positive scores above the cut-off value, compared with none of 9 sera from PF patients (0%), 1 of 72 sera from BP patients (1.4%), none from the DH patient, and none of 41 sera from patients with non-autoimmune bullous diseases (0%). Thus, the sensitivities of Dsg3 in PV and Dsg1 in PF were 85% and 100%, respectively. The specificities of Dsg3 in diagnosing PV and Dsg1 in PF were 99.1% and 97.4%, respectively. Additionally, the positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio in diagnosing PV were 0.94, 0.97, 96.9 and 0.151, respectively. The positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio in diagnosing PF were 0.75, 1.00, 38 and 0, respectively.

Among the 17 Dsg3 ELISA-positive PV patients, 11 patients had both oral mucosal and cutaneous involvement (named group 1), while 5 patients had only skin lesions (named group 2), and 1 had only oral mucosal involvement (named group 3). The average Dsg3 and Dsg1 values of group 1 patients were 164.13 ± 12.6 and 156.94 ± 21.8 , respectively. The average Dsg3 and Dsg1 values of group 2 patients were 95.64 ± 35.2 and 113.94 ± 16.8 , respectively. The Dsg3 and Dsg1 values of the group 3 patient were 37.6 and 30.7, respectively. The difference in Dsg1 ELISA titers between group 1 and group 2 patients was not significant ($p=0.142$). The difference in Dsg3 ELISA titers between group 1 and group 3 patients was also not significant ($p=0.126$).

ELISA scores to monitor disease activity

We used the dosage of systemic steroid as the indicator of disease activity. When the dosage was tapered, it meant clinical improvement of the disease. If the disease flared up, the dosage was increased. The correlation

between ELISA titers and prednisolone dosage in each patient was plotted according to the time course. The concomitantly used steroid-sparing agents were also plotted (Figures 1 and 2A). In PV patients 3, 4 and 5, the Dsg3 ELISA titers decreased in parallel with disease activity and increased when the patient had a flare-up. In PF patients, the Dsg1 ELISA titers fluctuated in parallel with disease activity over the time course. The correlation of average prednisolone dosage and average ELISA titers of 6 PV patients at each sampling time is also shown in Figure 2B. At the second to fourth samplings, ELISA Dsg3 titers fluctuated in parallel with prednisolone dosage. When generalized linear model repeated measurement method was used to determine the correlation between disease activity and ELISA Dsg3 titers, the difference in Dsg3 titers in the within-subjects analysis was of borderline significance ($p=0.057$), and the between-subjects analysis of Dsg3 titers showed a significant difference ($p=0.016$).

Discussion

We evaluated the Dsg1 and Dsg3 ELISA tests with a large number of serum samples obtained from patients with pemphigus, BP and patients with other blistering diseases. The male:female ratios in PV and PF patients were 7:3 and 2:1, respectively. This male predominance is not consistent with previous reports of equal sex distribution.¹⁶⁻¹⁹ However, because our hospital serves mainly veterans, the sex difference might not be meaningful.

In the present study, the sensitivity and specificity of desmoglein ELISAs were high and were consistent with previously reported data.⁷⁻¹² A positive Dsg3 result was indicative of PV, regardless of the associated Dsg1 result. A negative Dsg3 and a positive Dsg1 indicated a diagnosis of PF. Therefore, PV and PF could be distinguished by using a combination of Dsg1 and Dsg3 ELISA data.

We used these ELISAs to measure antibody levels of Dsg1 and Dsg3 and examined their correlation with the progression of disease activity in 7 pemphigus patients (6 PV, 1 PF). However, the within-subjects difference in ELISA Dsg3 titers in each PV patient showed borderline significance ($p=0.057$). Therefore, we could not determine whether desmoglein ELISAs would be a valuable tool for monitoring disease activity because of the small sample size in our study.

According to the Dsg compensation hypothesis,^{20,21} the clinical phenotype of pemphigus is determined by the underlying Dsg antibody profile²²⁻²⁴

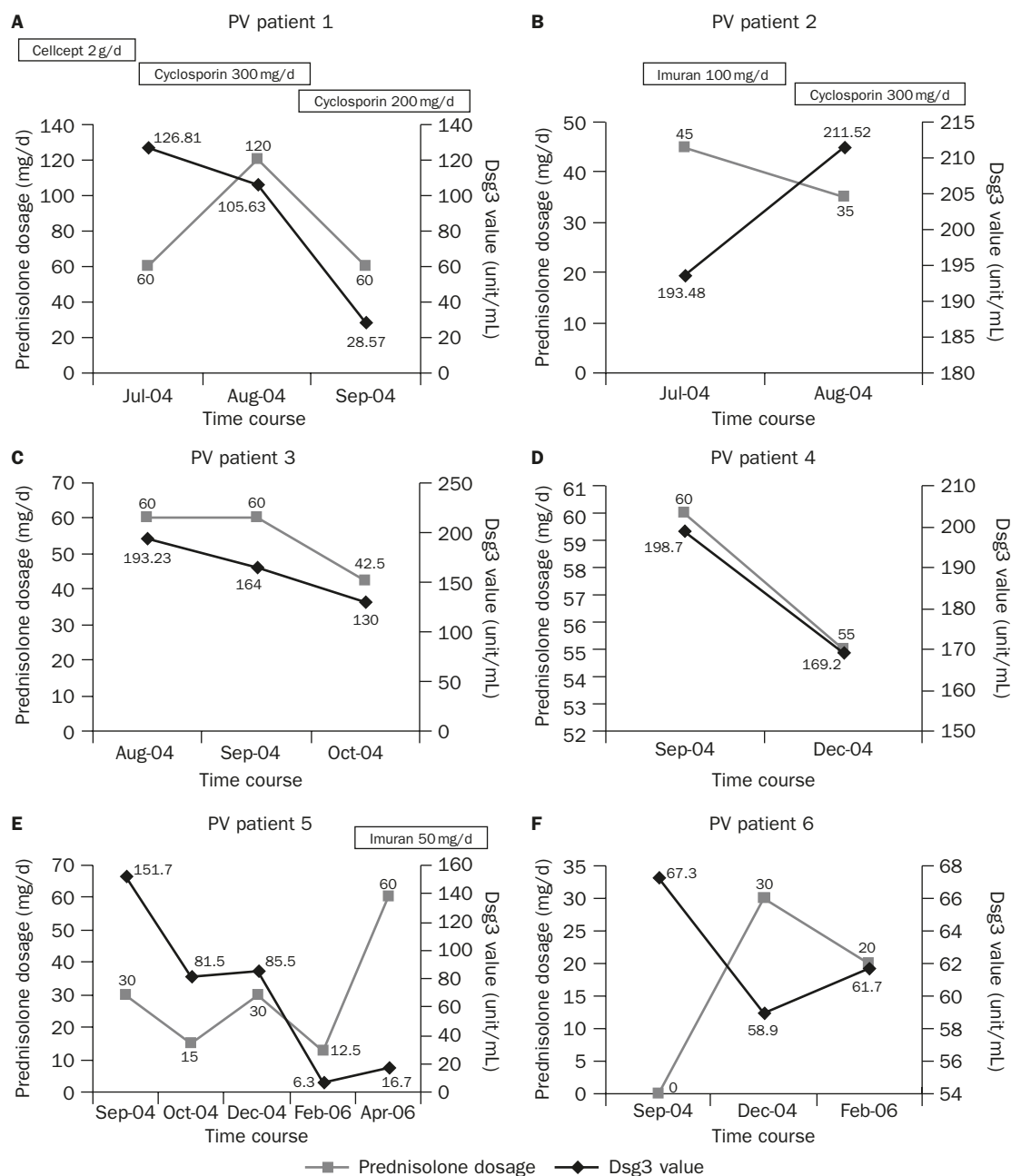


Figure 1. Correlation between desmoglein (Dsg)3 ELISA titers and prednisolone dosage of each pemphigus vulgaris (PV) patient (A–F) over a time course. The concomitantly used steroid-sparing agents are shown in boxes for each patient along the time course.

and by the tissue distribution of Dsg1 and Dsg3. Mucosal PV is seen only in patients with Dsg3 antibodies but no Dsg1 antibodies; PF is seen only in cases with Dsg1 antibodies but no Dsg3 antibodies. Mucocutaneous PV may occur in cases with Dsg3 antibodies alone, but severe cutaneous involvement is seen only if Dsg1 antibodies are also present. Harman et al have reported that the severities of cutaneous and oral pemphigus are related quantitatively to Dsg1 and Dsg3 antibodies, respectively.²⁵ In our study, both

the difference in Dsg1 ELISA titers between group 1 and group 2 patients and the difference in Dsg3 ELISA titers between group 1 and group 3 patients were not significant. Thus, our results did not support the previous report that the higher the Dsg ELISA titers, the more extensive the lesions. In addition, the patient who had only oral lesions had both positivity of Dsg1 and Dsg3. This was also not consistent with the previous report that mucosal PV is seen only in patients with Dsg3 antibodies but no Dsg1 antibodies.

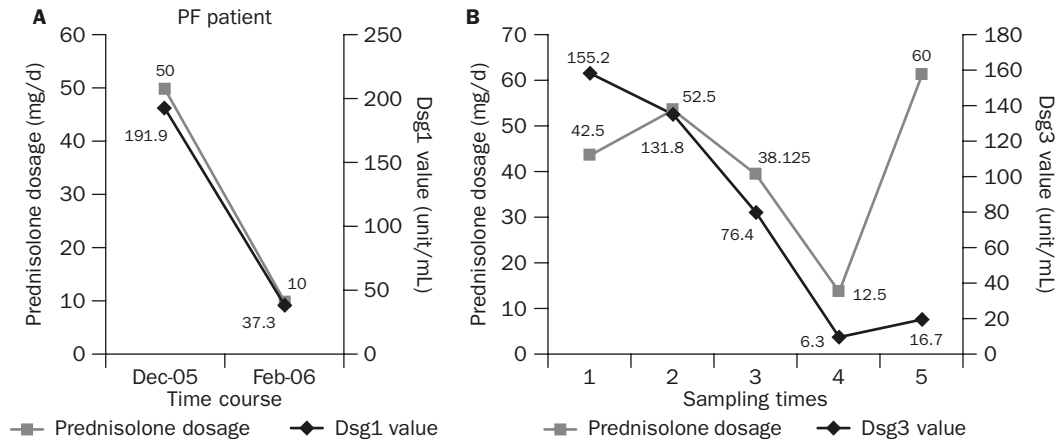


Figure 2. (A) Desmoglein (Dsg)1 ELISA titers tend to fluctuate in parallel with prednisolone dosage (disease activity) over a time course. (B) Correlation between average prednisolone dosage and average Dsg3 ELISA titers at different sampling times in 6 pemphigus vulgaris patients. PF=pemphigus foliaceus.

In future, the investigation of immunobullous disorders is likely to move towards antigen-specific techniques. The present study showed that Dsg1 and Dsg3 ELISAs could provide sensitive, specific, and reproducible data and allow correct diagnosis of PF and PV. However, more cases should be studied to determine whether Dsg1 and Dsg3 ELISAs are useful in monitoring the disease activity of pemphigus.

In conclusion, our results indicate that desmoglein ELISAs may serve as adjunct diagnostic tools for the initial diagnosis of pemphigus.

Acknowledgments

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