Evaluation of Sensitivity and Specificity of Enzyme-Linked Immunosorbent Assay (ELISA) for Detecting Antidesmoglein 1 and 3 in Thai Patients with Pemphigus Vulgaris and Foliaceus

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Objective: *Pemphigus is an acquired autoimmune blistering skin diseases, of which pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are two major subtypes. A novel commercial enzyme-linked immunosorbent assay (ELISA) against Dsg1 and Dsg3 has been well established for diagnosis and prediction of disease activity in PF and PV. At present, the benefit of anti-Dsg 1 and anti-Dsg 3 IgG by ELISA in the diagnosis of pemphigus in Thai patients has never been reported. The objective of the present study is to evaluate the sensitivity and specificity of ELISA for detecting antidesmoglein 1 and 3 in Thai patients with pemphigus.*
Material and Method: *Retrospective review of anti-Dsg1 and anti-Dsg3 antibody ELISA test results from 48 serum samples collected from 27 patients with PV, seven patients with PF, and 14 controls.*
Results: *The sensitivity of Dsg1 and Dsg3 ELISA for all patients with PV was 64% and 77.8% respectively. When subgrouped into only PV patients with new diagnosis, the sensitivity of Dsg 1 and Dsg 3 ELISA increased to 85.7% and 100%. In all PF patients, the sensitivity of anti-Dsg 1 ELISA was 71.4% and 100% for newly diagnosed PF cases. Anti-Dsg 3 was not detected in the PF group. The specificity of ELISA for anti-Dsg 1 and anti-Dsg 3 in both types of pemphigus was 85.7% and 92.3% respectively.*
Conclusion: *Dsg 1 and Dsg 3 ELISA is a simple, highly sensitive and specific test in Thai pemphigus patients with 100% sensitivity in the diagnosis of both new pemphigus vulgaris and foliaceus patients.*

Keywords: *Desmoglein 1, Desmoglein 3, Enzyme-linked immunosorbent assay, Pemphigus vulgaris, Pemphigus foliaceus*

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Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are two major subtypes of pemphigus[1]. Patients with PV have circulating IgG antibody targeting either the antigen desmoglein 3 (Dsg 3) for isolated mucosal involvement or both desmoglein 3 and desmoglein 1 (Dsg 3 and Dsg 1) for mucocutaneous involvement, while patients with PF have only circulating IgG antibody to Dsg 1[2-5]. The diagnosis of pemphigus is based on clinical manifestations, histologic findings and either direct immunofluorescence (DIF) or indirect immunofluorescence (IIF). Although immunofluorescence can be beneficial for identifying the circulating antibody-targeting antigen on the surface of keratinocytes in pemphigus, false-negative results may occur because of substrate sensitivity, technical error, and rarely, the prozone phenomenon[6]. In addition they are time consuming, and impractical for routine screening of large numbers of serum samples. Recently, a novel enzyme-linked immunosorbent assay

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(ELISA) against Dsg 1 and Dsg 3 has become commercially available and had been found to be extremely sensitive and specific[7-17]. At present, the sensitivity and specificity of anti-Dsg 1 and anti-Dsg 3 IgG by ELISA in the diagnosis of pemphigus in Thai patients have never been reported.

Material and Method

Subjects

All serum samples sent for ELISA test against Dsg 1 and Dsg 3 at Ramathibodi Hospital between February 2006 and November 2007 were retrospectively evaluated. During this period, 53 serum samples were collected from 39 pemphigus patients (PV and PF) and 14 patients with other dermatologic disorders.

The pemphigus patients were included in the present study only if they met all of the following diagnostic criteria of PV or PF: clinical manifestations and histopathology characteristic of PV/PF, and positive direct or indirect immunofluorescence. Five of the 39 pemphigus patients did not meet the diagnostic criteria of PV/PF and were excluded from the study. Therefore, only 48 serum samples were included in the present study, 27 patients with PV, seven patients with PF, and 14 patients with other dermatologic disorders. The 14 patients were assigned to be the control group.

The control group comprised of exfoliative dermatitis (n = 2), aphthous ulcer (n = 2), Sweet's syndrome (n = 2), systemic lupus erythematosus (n = 2), Stevens-Johnson syndrome and toxic epidermal necrolysis overlap (n = 1), drug eruption (n = 1), pseudo-ocular cicatricial pemphigoid (n = 1), leukoplakia (n = 1), idiopathic thrombocytopenic purpura (n = 1), and erythema multiforme (n = 1).

The present study was conducted under the approval of Ethical Clearance Committee (Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand).

Indirect immunofluorescence

Normal human skin was used as a substrate for indirect immunofluorescence to detect intercellular antibody titer in pemphigus.

Dsg 1 and Dsg 3 ELISA

Sera were stored at -70°C until the assays were performed. The ELISAs were performed with 1:100 dilution of serum using MESACUP desmoglein test kits (Medical & Biological Laboratories Co. Ltd., Nagoya, Japan) following the manufacturer's instructions. Anti-Dsg 1 and anti-Dsg 3 values above 14 Unit/ml and 7 Unit/ml were considered positive.

Statistical analysis

All statistical demographic data are presented as mean $\pm$ Standard error of the mean (SEM), range. The sensitivity and specificity of Anti-Dsg 1 and 3 ELISA between each group were determined statistically by Chi-Square test.

Results

In the present study, pemphigus patients were divided into three groups, group 1 included all PV or PF patients including cases in remission, active disease, and newly diagnosed cases, group 2 included patients with active disease and newly diagnosed cases, and group 3 were only newly diagnosed cases.

The average ages of PV patients, PF patients, and controls were 47.04 years (range 22-80), 48.0 years (range 25-64), and 55.14 years (range 32-90), respectively. The male and female ratios in PV, PF, and controls were 1:3.5, 1:1.3, and 1:2.5 respectively as shown in Table 1.

The sensitivity and specificity of the ELISA in pemphigus patients is shown in Table 2 and 3.

In order to calculate sensitivity and specificity of Dsg 1 and Dsg 3 ELISA, each subgroup of both diagnosed PV and PF was compared with controls and other dermatoses. For Dsg 1 sensitivity it was only 64% in overall PV cases. It increased up to 76.2% in group of active and newly diagnosed PV individuals. Nevertheless, the higher sensitivity (85.7%) was revealed in subgroup of firstly diagnosed PV.

On the other hand, Dsg 1 sensitivity for overall PF subjects was 71.4%. Interestingly, it achieved 100% in both groups of active and newly diagnosed patients and in subgroup of firstly identified PF. Since

Table 1. Patients' characteristics

Diagnosis	Serum samples (n = 48)	Male/ female	Mean age $\pm$ SD (min-max)
Pemphigus vulgaris			
All	27	6/21	47.04 $\pm$ 13.3 (22-80)
Active disease	15		
New Cases	8		
Pemphigus foliaceus			
All	7	3/4	48.00 $\pm$ 13.3 (25-64)
Active disease	3		
New cases	2		
Controls	14	4/10	55.14 $\pm$ 17.6 (32-90)

Table 2. Summary of data in pemphigus vulgaris

Subgroup (n)	Dsg	Dsg results	PV	Controls	Sensitivity	Specificity	+LLR	-LLR
Gr I: All PV (n = 27)	Dsg 1	+	16	2	64.0%	85.7%	4.48	0.42
		−	9	12				
	Dsg 3	+	21	1	77.8%	92.3%	10.11	0.24
		−	6	12				
Gr II: Active + New cases (n = 23)	Dsg 1	+	16	2	76.2%	85.7%	5.33	0.28
		−	5	12				
	Dsg 3	+	20	1	87.0%	92.3%	11.30	0.14
		−	3	12				
Gr III: New cases (n = 8)	Dsg 1	+	6	2	85.7%	85.7%	6.00	0.17
		−	1	12				
	Dsg 3	+	8	1	100.0%	92.3%	13.00	0
		−	0	12				

* Positive titer for anti-Dsg 1 > 14 Unit/ml
** Positive titer for anti-Dsg 3 > 7 Unit/ml

Table 3. Summary of data in pemphigus foliaceus

Subgroup (n)	Dsg	Dsg results	PV	Controls	Sensitivity	Specificity	+LLR	-LLR
Gr I: All PF (n = 7)	Dsg 1	+	5	2	71.4%	85.7%	5.0	0.33
		−	2	12				
	Dsg 3	+	0	1	-	92.3%	0	1.08
		−	6	12				
Gr II: Active + New cases (n = 5)	Dsg 1	+	5	2	100.0%	85.7%	7.0	0
		−	0	12				
	Dsg 3	+	0	1	-	92.3%	0	1.08
		−	4	12				
Gr III: New cases (n = 2)	Dsg 1	+	2	2	100.0%	85.7%	7.0	0
		−	0	12				
	Dsg 3	+	0	1	-	92.3%	0	1.08
		−	2	12				

* Positive titer for anti-Dsg 1 > 14 Unit/ml
** Positive titer for anti-Dsg 3 > 7 Unit/ml

Dsg 3 is predominated in mucosal areas, it is commonly negative in PF. Similarly, Dsg 3 was totally negative in all PF subjects of the present study. As a result, the sensitivity of Dsg 3 could not be calculated in PF. In contrary, the sensitivity of ELISA detecting antibody against Dsg 3 showed 77.8% in all PV patients. It excelerayed to 87% and 100% in active and newly diagnosed individuals and in the subgroup of firstly diagnosed PV, respectively. Because of using the same control group, calculated results of the specificity of Dsg 1 and Dsg 3 ELISAs in each PV and PF subgroups were similar, 87.5% and 92.3%, respectively.

Since this was a retrospective study, the collected data were incomplete in blood samplings, as demonstrated in the followings. In PV group, two patients (1 active and 1 newly diagnosed case) were not tested for anti-Dsg 1 but tested for only anti-Dsg 3. In PF group, one active case was also not collected for anti-Dsg 3. Finally in the control group, one case diagnosed ITP was not collected for Anti-Dsg 3.

Discussion

To the best of the authors' knowledge, this is the first report of sensitivity and specificity of ELISA

for detecting anti-Dsg 1 and 3 in Thai patients with pemphigus vulgaris and foliaceus. The present study supported the previous studies that ELISA for anti-Dsg 1 and 3 is a sensitive and specific tool for diagnosis of pemphigus[7-17] as summarized in Table 4.

Serum samples from newly diagnosed patients (group 3) for both the Dsg 1 and Dsg 3 ELISA showed a sensitivity of 100% for the diagnosis of PF and PV. Dsg 3 autoantibodies were not detected in any of the PF subjects while Dsg1 autoantibodies were detected in about 60% of all PV subjects. The presence of Dsg 1 autoantibodies in more than 50% of PV cases were reported by previous studies[18,19] and appeared to be associated with mucocutaneous PV. Anti-Dsg 1 and 3 ELISA technique is a beneficial adjuctive tool to diagnosis subtypes of pemphigus[5,20-22]. A positive anti-Dsg 1 and 3 ELISA is a marker of PF and PV, respectively. This technique is more advantageous than immunoblotting and immunoprecipitation as it is not only simple, allowing the analysis of large numbers of samples in a relatively shorter time, but it also yields high sensitivity and high specificity results for PV and PF, also the data of ELISA test is objective and quantitative as numerical value from continuous scales while IIF is subjective in which results are interpreted from a series of discontinuous serum dilutions. In addition, the ELISA value can be used to monitor disease activity[23,24].

There is a variation in the range of the cut off index values of Dsg ELISA kit in different labs. The positive cut off values used in Ramathibodi Hospital were above 14 IU/ml for Dsg 1 and above 7 IU/ml for Dsg 3, which were equal or lower than previous studies (Dsg 1 range: 14-30 IU/ml, Dsg 3 range: 7-40 IU/ml)[5,7,12-17,25,26]. The specificity of ELISA for anti-Dsg 1 and anti-Dsg 3 in pemphigus was 85.7% and 92.3% respectively. In the present study, false positive anti-Dsg 1 or 3 antibody were seen in three patients without pemphigus phenotypes, two patients with SLE (anti-Dsg 1) and one psoriasis patient with exfoliative dermatitis (anti-Dsg 3). This was also seen in previous studies[10,12,20]. They reported the presence of pemphigus autoantibodies in sera of healthy controls, patients with autoimmune connective tissue diseases, and bullous pemphigoid etc. Therefore, further studies should be performed to explain this phenomenon.

The evaluation of the correlation between disease activity and desmoglein antibody titer in the present study was limited by the small sample size and retrospective study. In addition, control cases are too small, they could affect the results of specificity of the test. Further studies, incorporating a large number of patients evaluated in a prospective manner is essential to provide additional information.

Conclusion

Dsg1 and Dsg3 ELISA provide a simple, highly sensitive, and specific test that can be used as a useful adjunctive tool to aid the diagnosis of pemphigus especially in new cases. However, more cases and

Table 4. Previous studies show the sensitivity and specificity of Dsg 1 and 3 ELISA in PF and PV

Study	Number	PV Sensitivity of Dsg 3 (%)	PV Specificity of Dsg 3 (%)	PF Sensitivity of Dsg 1 (%)	PF Specificity of Dsg 1 (%)
Haung 2007[17]	114 Controls 20 PV 9 PF	85	99.1	100	97.4
Harmann 2000[12]	317 Controls 82 PV 25 PF	95	>98	92	>98
Amagai 1999[7]	179 Controls 81 PV 48 PF	85.2	100	89.6	99.4
Ishii 1997[8]	76 Controls 49 PV 46 PF	94	96	96	96
Our study (In Gr 2 which excluded cases in remission)	14 Controls 23 PV 5 PF	87	92.3	100	85.7

PV = pemphigus vulgaris, PF = pemphigus foliaceus

further prospective studies should be done to determine whether Dsg1 and Dsg 3 ELISA values are useful in monitoring the disease activity of pemphigus.

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การประเมินผลความไวและความจำเพาะของการตรวจหาแอนติบอดิต่อ desmoglein 1 และ 3 โดยวิธี ELISA ผู้ป่วยโรค Pemphigus vulgaris และ Pemphigus foliaceus ในคนไทย

สุธินี กุลกลการ, เพ็ญพรรณ วัฒนไกร, วาสนภ วชิรมน, ปาริชาต ชลิดาพงศ์

วัตถุประสงค์*: Pemphigus*เป็นกลุ่มของโรคภูมิแพ้ตุ่มน้ำประกอบด้วย 2 กลุ่มหลักๆ คือ *Pemphigus vulgaris (PV)* และ *Pemphigus foliaceus (PF)* ในผู้ป่วย *PV* จะมี *IgG* แอนติบอดิ ต่อแอนติเจนบนผิวของเซลล์ *desmoglein 3 (Dsg 3)* หรือ ทั้ง *desmoglein 3 (Dsg 3)* และ *desmoglein 1 (Dsg 1)* ส่วนผู้ป่วย *PF* จะมี *IgG* แอนติบอดิ ต่อ *Dsg 1* ปัจจุบันมีการใช้วิธี *ELISA* ตรวจหาแอนติบอดิต่อ *Dsg 1* และ *Dsg 3* ในผู้ป่วยโรค *Pemphigus* ทั้ง 2 ชนิดนี้ แต่ยังไม่เคยมีการศึกษาถึงประโยชน์ของการตรวจในคนไทยมาก่อน จุดประสงค์ของการศึกษานี้เพื่อประเมินผลความไว และความจำเพาะของการตรวจหาแอนติบอดิต่อ *desmoglein 1* และ *3*โดยวิธี *ELISA* ผู้ป่วย *Pemphigus* ในคนไทย

วัสดุและวิธีการ*: การศึกษาย้อนหลัง รวบรวมตัวอย่างซีรัมส่งตรวจหาแอนติบอดิต่อ Dsg 1 และ 3 โดยวิธี ELISA จากผู้ป่วยทั้งหมด 48 คน จำแนกเป็น PV 27 คน PF 7 คน และกลุ่มเปรียบเทียบ 14 คน ที่เป็นโรคผิวหนังอื่น ๆ*

ผลการศึกษา*: ความไว ELISA ต่อ Dsg 1 และ 3 ของผู้ป่วยทั้งหมดที่เป็นโรค PV คือ 64% และ 77.8% ตามลำดับ, และเมื่อแบ่งเป็นผู้ป่วย PV ที่ได้รับการวินิจฉัยโรคครั้งแรก ความไวเพิ่มขึ้นเป็น 85.7% และ 100% สำหรับผู้ป่วยทั้งหมดที่เป็นโรค PF พบความไวของวิธี ELISA ต่อ Dsg 1 เป็น 71.4% และเพิ่มขึ้นเป็น 100% ในผู้ป่วย PF ที่ได้รับการวินิจฉัยโรคครั้งแรกเช่นกัน ไม่พบผลบวกของแอนติบอดิต่อ Dsg 3 ในผู้ป่วย PF ความจำเพาะของ ELISA ต่อ Dsg 1 และ 3 ในผู้ป่วย Pemphigus ทั้ง 2 ชนิด คือ 85.7% และ 92.3% ตามลำดับ*

สรุป*: การตรวจหาแอนติบอดิต่อ Dsg 1 และ Dsg 3 โดยวิธี ELISA เป็นวิธีที่ง่าย มีความไวและความจำเพาะสูงในการวินิจฉัยผู้ป่วยโรค PV และ PF ในคนไทย โดยเฉพาะการวินิจฉัยผู้ป่วยใหม่*