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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Objectives: Pemphigus is an acquired autoimmune blistering skin disease, 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-Dsg1 and anti-Dsg3 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 Dsg1 and Dsg3 ELISA increased to 85.7% and 100%. In all PF patients, the sensitivity of anti-Dsg1 ELISA was 71.4% and 100% for newly diagnosed PF cases. Anti-Dsg3 was not detected in the PF group. The specificity of ELISA for anti-Dsg1 and anti-Dsg3 in both types of pemphigus was 85.7% and 92.3% respectively.

Conclusion: Dsg1 and Dsg3 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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(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), Stevens-Johnson syndrome and toxic epidermal necrolysis overlap (n = 1), drug eruption (n = 1), pseudocutaneous 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 ± 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.

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

Table 1. Patients’ characteristics
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(5-17) 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 adjunctive 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**

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

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

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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References

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

สุธินี กุลกลการ, เพ็ญพรรณ วัฒนไกร, วาสนภ วชิรมน, ปราชาวดี ชลิดาพงศ์

วัตถุประสงค์: Pemphigus เป็นกลุ่มของโรคภูมิแพ้ตุ่มน้ำประกอบด้วย 2 กลุ่มหลักๆ คือ Pemphigus vulgaris (PV) และ Pemphigus foliaceus (PF) ในผู้ป่วย PV จะมี IgG แอนติบอดิต่อ desmoglein 3 (Dsg 3) หรือทั้ง desmoglein 3 (Dsg 3) และ desmoglein 1 (Dsg 1) ส่วนผู้ป่วย PF จะมี IgG แอนติบอดิต่อ desmoglein 1 (Dsg 1) และ 3 โดยวิธี ELISA ผู้ป่วย Pemphigus ในคนไทย

วัสดุและวิธีการ: การศึกษาเป็น前瞻 รวบรวมตัวอย่างซีรัมส่งตรวจหานอนติบอดิต่อ desmoglein 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, และมีนักแบ่งเป็นผู้ป่วย PV ที่ได้รับการวินิจฉัยโรคครั้งแรก ความไวเพิ่มขึ้นเป็น 85.7% และ 100% สำหรับผู้ป่วยทั้งหมดที่เป็นโรค PF พบความไวของ ELISA ต่อ Dsg 1 เป็น 71.4% และเพิ่มขึ้นเป็น 100% ในผู้ป่วย PF ที่ได้รับการวินิจฉัยโรคครั้งแรก ความจำเพาะของ ELISA ต่อ Dsg 1 และ 3 ในผู้ป่วย Pemphigus ทั้ง 2 ชนิด คือ 85.7% และ 92.3% ตามลำดับ

สรุป: การตรวจหานอนติบอดิต่อ desmoglein 1 และ 3 โดยวิธี ELISA เป็นวิธีที่ง่าย มีความไวและความจำเพาะสูงในการวินิจฉัยผู้ป่วยโรค PV และ PF ในคนไทย โดยเฉพาะการวินิจฉัยผู้ป่วยใหม่