Correlation Between Total Serum IgE Levels and Some Characteristics in Vietnamese Pemphigoid Patients

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Summary

Objectives: To determine the correlation between total serum IgE levels and some clinical and subclinical characteristics in pemphigoid patients.

Methods: A cross-sectional study on 42 pemphigoid patients, all of which were newly diagnosed. Information was collected from patient interviews and medical records. Total serum IgE levels were determined using a total IgE ELISA following the manufacturer’s instructions. Eosinophil counts were analyzed on the same day when serum samples were taken. For IgE DIF staining of perilesional skin, IgE Goat anti-Human, FITC was used, incubation was performed overnight at 4°C.

Results: Among 42 pemphigoid patients, the average age was 73.2 ± 15.5 years old. 60% of pemphigoid patients had elevated total serum IgE levels, the mean value was 121.6 ± 101.8 U/mL. Total serum IgE levels were significantly correlated with eosinophil counts (r = 0.61; p < 0.05) and the BPDAI urticaria/erythema (U/E) score (r = 0.50; p < 0.05). However, total circulating IgE levels were not significantly associated with total BPDAI score (p > 0.05). DIF IgE staining on perilesional skin of pemphigoid patients had a positive rate of 14.3%.

Conclusion: Total serum IgE values correlated with some clinical and subclinical characteristics in pemphigoid patients. IgE autoantibodies were present in the skin of pemphigoid patients, supporting the hypothesis that IgE potentially modulates pruritus associated with pemphigoid.

Keywords: IgE, pemphigoid, eosinophil.

1. Introduction

Pemphigoid is a common disease in the group of autoimmune bullous diseases. Its clinical manifestations often include pruritus and/or cutaneous tense blisters. Although its prevalence in the population is relatively low, this disease can cause many serious complications. Bullous pemphigoid (BP) is chronic and persistent with multiple episodes of spontaneous exacerbation and remission, however, there is an increased mortality in patients with prolonged illnesses, especially in immunocompromised or debilitated patients. Moreover, the use of corticosteroids in the treatment of these diseases also causes side effects at different extent. Therefore, it is necessary to make early diagnosis and timely treatment, as well as to carefully study the pathogenesis and prognostic factors, in order to improve the outcome of this disease.

Current diagnostic criteria of pemphigoid include both clinical and laboratory criteria, with the important role of detecting autoantibodies in patients’ skin and serum, among which, the most...
common is IgG autoantibodies\textsuperscript{5,6}. However, there have been reports of increased total serum IgE levels in pemphigoid patients, as well as detection of specific IgE autoantibodies in the serum and IgE deposition along basement membrane zone on DIF staining in patients with pemphigoid\textsuperscript{7-13}. These studies have shown that IgE autoantibodies play a certain role in the pathogenesis of pemphigoid (associated with pruritus)\textsuperscript{7}. This gave light to a novel treatment therapy for pemphigoid disease, which is omalizumab (an anti-IgE monoclonal antibody), especially in patients with contraindications, recalcitrance or intolerance to corticosteroids. There is increasing evidence for the efficacy and safety of omalizumab in the treatment of recalcitrant pemphigoid\textsuperscript{14}. Currently, in Vietnam, there has not been any study on the total serum IgE level in pemphigoid patients. Therefore, this study was conducted to determine the correlation between total serum IgE level and some clinical and subclinical characteristics in pemphigoid patients at the National Hospital of Dermatology and Venereology (NHDV), 2021 - 2022.

2. MATERIALS AND METHODS

2.1. Selection of patients

2.1.1. Selection criteria: Newly diagnosed pemphigoid patients at the National Hospital of Dermatology and Venereology from July 2021 to September 2022.

2.1.2. Exclusion criteria: For total serum IgE levels determining, eosinophil counts and IgE DIF staining we excluded

- Patients with asthma, allergic rhinitis, or atopic dermatitis.
- Patients with other skin diseases that cause pruritus, such as acute urticaria, scabies, etc.
- Patients with a history of taking systemic corticosteroids or H1 antihistamines within 3 weeks previous to the study.

2.2. Methods

2.2.1. Study design: Descriptive cross-sectional study.

2.2.2. Sample

To investigate the elevated rate of total serum IgE among pemphigoid patients, WHO estimation formula of descriptive study was applied:

\[ n = Z^{2}_{1-\alpha/2} \frac{p(1-p)}{(p.e)^2} \]

- \( n \): sample size.
- \( p \): anticipated population proportion of patients with elevated total serum IgE level.
- \( Z_{1-\alpha/2} \): Confidence interval (\( \alpha = 0.05 \)): 95%, \( Z = 1.96 \).
- \( e \): relative precision, \( e = 0.3 \).

In this study, we chose \( p = 59.3\% \) as the anticipated population proportion of pemphigoid patients with elevated total serum IgE level. When applied to the formula, \( n = 30 \).

From July 2021 to September 2022, 42 newly diagnosed pemphigoid patients at the National Hospital of Dermatology and Venereology participated in the study. Convenience sampling was used. 7 pemphigoid patients were excluded from total serum IgE levels measurement, eosinophil counts and DIF IgE staining due to history of taking systemic corticosteroids or H1 antihistamines within 3 weeks previous to the study.

2.2.3. Study procedure

Patients were diagnosed pemphigoid if three diagnostic criteria were met: (1) compatible clinical features of pruritus and/or cutaneous blisters, (2) positive linear IgG staining by direct immunofluorescence (DIF) microscopy and (3) positive IgG staining on the epidermal side of salt-split skin by in DIF microscopy\textsuperscript{5,6}. 

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Disease severity was assessed by the BPDAI score.

Total serum IgE levels were determined using a Total-IgE ELISA Test Kit (IBL International GmbH, Germany) following the manufacturer’s instructions, BioTek ELx50 self-contained automated strip washer, ELx808 Absorbance Microplate Reader (normal IgE range < 100 UI/mL).

Eosinophil counts were analyzed during routine laboratory testing on the same day when serum samples were taken (normal range 0.0 - 0.8 G/L).

For DIF staining of IgE, skin biopsy specimens obtained from perilesional skin were transported and embedded in Shandon Cryomatrix Gel (Thermo Fisher Scientific, Massachusetts, USA) and stored at -80°C until further processing. Cryosections of the skin biopsies obtained from Thermo Fisher Scientific - Cryotome were stained using Polyclonal Goat anti-Human IgE Secondary Antibody, FITC (affinity purified and heavy chain specific, diluted 1:100 with PBS buffer solution; Invitrogen, Thermo Fisher Scientific). Incubation was performed overnight at 4°C. After washing, the slides were coverslipped with a mounting medium (Thermo Fisher Scientific).

Other information was collected from patient interviews and medical records.

2.3. Statistical analysis

For statistical analysis, we used SPSS 20.0 software. Chi-square tests or Fisher’s exact tests, non-parametric Mann-Whitney U-test and Kruskal-Wallis were used to determine statistical significance between groups. Spearman’s rank correlation coefficient (r) was used to determine the statistical dependence between variables. The p-value of < 0.05 was considered statistically significant.

2.4. Ethical consideration

Participants were explained about the purpose of the study, they voluntarily agreed to participate. Data was kept confidential and used for research purposes only. The study was approved by the Ethics Committee of the NHDV.

3. RESULTS

3.1. Patients clinical and subclinical characteristics

Table 1. Patients clinical and subclinical characteristics (n = 42)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Count (n)</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group (years old)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50</td>
<td>3</td>
<td>7.1</td>
</tr>
<tr>
<td>50 - 69</td>
<td>15</td>
<td>35.7</td>
</tr>
<tr>
<td>70 - 89</td>
<td>18</td>
<td>42.9</td>
</tr>
<tr>
<td>≥ 90</td>
<td>6</td>
<td>14.3</td>
</tr>
<tr>
<td>Mean age (year) (± SD)</td>
<td>73.2 ± 15.5</td>
<td></td>
</tr>
<tr>
<td>Mean age of male patients (± SD)</td>
<td>71.4 ± 16.7</td>
<td>p = 0.198</td>
</tr>
<tr>
<td>Mean age of female patients (± SD)</td>
<td>78.4 ± 10.4</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31</td>
<td>73.8</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>26.2</td>
</tr>
<tr>
<td>Eosinophils (n = 35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>16</td>
<td>45.7</td>
</tr>
<tr>
<td>Elevated</td>
<td>19</td>
<td>54.3</td>
</tr>
</tbody>
</table>
Among 42 newly diagnosed pemphigoid patients, 31 were male (73.8%) and 26.2% were female.

Patients had an average age of 73.2 ± 15.5 years old, with no statistically significant difference between male and female. Age group 70 - 89 years old accounted for the highest proportion (42.9%).

Elevated eosinophils were observed in 19 of 35 patients (54.3%).

Among 35 patients who fitted in the selection criteria for IgE measurement, 21 patients (60%) had elevated total serum IgE level. Mean total serum IgE levels among pemphigoid patients was presented in Table 2.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Count (n)</th>
<th>Mean total serum IgE level (IU/mL) (± SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>25</td>
<td>108.2 ± 87.6</td>
<td>0.339</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>155.1 ± 130.2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>121.6 ± 101.8</td>
<td></td>
</tr>
</tbody>
</table>

The mean total serum IgE level among pemphigoid patients was 121.6 ± 101.8 UI/mL. There was no statistically significant difference (p > 0.05) in mean total serum IgE level between male and female patients.

### 3.2. Correlation between total serum IgE levels and some clinical and subclinical characteristics in pemphigoid patients

#### Eosinophil count

Figure 1. Correlation between peripheral eosinophil counts and total serum IgE levels in pemphigoid patients

Total serum IgE levels in pemphigoid patients were positively correlated with eosinophil counts, correlation coefficient \( r = 0.61; \) p < 0.05. An increased eosinophil count is associated with an increased total IgE value.
Figure 2. Correlation between total serum IgE levels and total BPDAI score in pemphigoid patients

Higher total serum IgE level was not significantly associated with a higher total BPDAI score ($r = 0.37; \text{Coef} = 0.1; p > 0.05$)

Figure 3. Correlation between total serum IgE levels and BPDAI urticaria/erythema (U/E) score in pemphigoid patients

$r = 0.50; \text{Coef} = 0.07; p < 0.05$
The score of urticaria and erythema (U/E) in the BPDAI scale had a positive correlation with the total IgE concentration in the blood, correlation coefficient \( r = 0.50 \) (\( p < 0.05 \)). Elevated total blood IgE levels were associated with increased U/E score in the BPDAI score of pemphigoid patients.

### 3.3. Immunofluorescent IgE staining of skin biopsies

Skin biopsies taken for DIF microscopy in 35 pemphigoid patients without medical history of systemic corticosteroids or H1 antihistamines within 3 weeks previous to the study were additionally stained for IgE. IgE linear deposits along the basement membrane zone were observed in 5 cases (14.3%).

![Image 1. Linear deposits of IgE were found by direct immunofluorescence microscopy of perilesional skin in 5 patients](image)

| Table 3. Associations between total serum IgE level, peripheral eosinophil counts and IgE DIF staining |
|---|---|---|---|
| | IgE DIF staining (+) (n = 5) | IgE DIF staining (-) (n = 30) | p |
| Mean serum IgE levels (IU/ml) | 153.4 ± 151.7 | 116.3 ± 93.2 | 0.604 |
| Mean eosinophil counts (G/L) | 3.8 ± 3.1 | 1.3 ± 1.5 | 0.021 |

There was no statistically significant association between total serum IgE level and the deposition of IgE along the basement membrane zone (\( p > 0.05 \)). However, higher eosinophil counts were significantly associated with positive IgE staining (\( p < 0.05 \)).

### 4. DISCUSSION

In this study, mean age of pemphigoid patients was 73.2 ± 15.5 years old, which were similar to previous studies at NHDV\(^{15,16}\). Eosinophilia was observed in 54.3% of pemphigoid patients, similar to the findings of Lamberts\(^7\), Kridin\(^{17}\). Eosinophilia and eosinophil infiltrations of dermis in pemphigoid patients are explained by the role of this leukocyte in the pathogenesis of the disease, via granulation release, chemotaxis, and increased production of MMP-9\(^{18}\). Messingham\(^{19}\) also reported that there may be a certain relationship between eosinophil levels and the severity of pemphigoid.

The mean total serum IgE level in the pemphigoid group was 121.6 ± 101.8 UI/ml. The median IgE concentration in Lamberts’ study was 164 UI/ml, similar to the results of our study, which used the same ELISA technique to quantify serum IgE levels. The elevation of serum IgE in pemphigoid patients could be explained by the role of IgE autoantibodies in the pathogenesis of the disease, in addition to the roles of IgG autoantibodies and complement\(^7,8\).

Our study showed that there was a positive correlation between eosinophil counts and total IgE concentrations, \( r = 0.61 \) (\( p < 0.05 \)) in pemphigoid patients, this result was similar to the study by Messingham\(^{19}\). In pemphigoid patients, eosinophils
increased expression of FcεRI, which is a receptor with high affinity for IgE\(^9\). This observation contributed to the association between eosinophilia and elevated total serum IgE level.

There was a positive correlation between the total serum IgE levels and BPDAI urticaria/erythema (U/E) score \((r = 0.50, p < 0.05)\) among 35 patients in our study. This result was similar to van Beek’s study which sample size was 52 patients\(^8\). However, Cozzani’s study among 32 patients\(^10\) showed that the total serum IgE values were higher in patients with mainly bullae than patients with mainly urticaria lesions; and total IgE serum levels were rather unrelated to disease severity. Our study also showed that there was no positive correlation between total IgE concentrations and total BPDAI score \((r = 0.37, p > 0.05)\). This may be explained by the role of IgG and complement autoantibodies in the pathogenesis of pemphigoid in addition to IgE autoantibodies, and the exact role of IgE is still not well understood.

IgE linear deposits along the basement membrane zone were observed in 5/35 cases (14.3%). The positive rates varied widely: 0%, 3%, 7%, 18%, 25% and 44% as reported by several previous studies\(^7,9-13\). Different staining methods and biopsy sites may explain these different results. In our study, among 5 patients with positive IgE staining, there are 4 patients with severe and 1 patient with moderate disease activity, according to BPDAI score. This group showed no statistically significantly higher circulating IgE levels, but had a higher number of eosinophils than the negative IgE staining group \((p < 0.05)\).

Due to time limit, this study did not recruit any nonbullous pemphigoid patients. Total serum IgE levels were not re-assessed after remission. Further studies should be done to investigate the correlation between specific serum IgE antibodies and clinical, subclinical characteristics in pemphigoid patients.

5. CONCLUSION

Among 42 pemphigoid patients, mean total serum IgE value was \(121.6 \pm 101.8\) UI/ml. 60% of pemphigoid patients had elevated total serum IgE levels. Total serum IgE levels were significantly correlated with eosinophil counts \((r = 0.61; p < 0.05)\) and the BPDAI urticaria/erythema (U/E) score \((r = 0.50; p < 0.05)\). However, total circulating IgE levels were not significantly associated with total BPDAI score \((p > 0.05)\). IgE DIF staining on perilesional skin of pemphigoid patients had a positive rate of 14.3%. These findings supported the hypothesis that IgE potentially modulates pruritus associated with pemphigoid.

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