Comparative study of indirect immunofluorescence, enzyme-linked immunosorbent assay, and the Tzanck smear test for the diagnosis of pemphigus

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BACKGROUND: Pemphigus is one of the potentially fatal autoimmune blistering diseases. An early and accurate diagnosis is important for prognosis and therapy. It may be difficult to diagnosis based on clinical grounds alone. Direct and indirect immunofluorescence, enzyme-linked immunosorbent assay, the Tzanck smear test, or histopathology are all available for the diagnosis of pemphigus. However, there are no generally accepted diagnostic criteria for the diagnosis of this condition at present.

OBJECTIVE: To evaluate the diagnostic value of indirect immunofluorescence, enzyme-linked immunosorbent assay, and the Tzanck smear test for the diagnosis of pemphigus in dental clinics.

METHODS: A single-center retrospective study was conducted, and the clinical data of 33 patients with pemphigus and 61 controls were collected and analyzed from the Department of Oral Medicine, Peking University School of Stomatology, during 2010–2014. The sensitivities and specificities of indirect immunofluorescence, enzyme-linked immunosorbent assay, and the Tzanck smear test were calculated and compared in two groups.

RESULTS: Sensitivities for the Tzanck smear test, indirect immunofluorescence, and enzyme-linked immunosorbent assay were 96.7%, 84.8%, and 84.8%, respectively, whereas the specificities of these tests were 60%, 91.8%, and 96.7%, respectively. The serial tests for the Tzanck smear test and enzyme-linked immunosorbent assay showed 82% sensitivity and 98.7% specificity.

CONCLUSIONS: The serial test for the Tzanck smear test and enzyme-linked immunosorbent assay may represent a simple, rapid, and reliable way to definitive diagnosis of pemphigus. It is recommended as a common test for the diagnosis of pemphigus in dental clinics.

Keywords: enzyme-linked immunosorbent assay; indirect immunofluorescence; pemphigus; serial test; Tzanck smear test

Introduction

Pemphigus is a rare, chronic, and potentially life-threatening autoimmune bullous disease characterized by widespread blistering and erosions on the skin and mucous membranes (1, 2). The incidence of pemphigus varies among different reports and geographic locations, which was reported ranged from 0.6 to 6.8 cases per million persons per year (3). However, no data are available on the incidence of pemphigus in China up to now (4).

Pemphigus is classified into subtypes based on the main autoantigens involved and the clinical manifestations. Pemphigus vulgaris (PV) is the most common form, accounting for nearly 80% of cases (5). As the early lesions of most PV patients are associated with the oral mucosa, most of these patients initially present to a dental clinic. Therefore, stomatologists play an important role in diagnosing and treating bullous diseases, and the convenient and reliable clinical diagnostic procedure is of importance in dental clinic.

Many tests are used to diagnose pemphigus, including direct immunofluorescence (DIF), indirect immunofluorescence (IIF), enzyme-linked immunosorbent assay (ELISA), the Tzanck smear test, and histopathology. There are no generally accepted diagnostic criteria for the diagnosis of this condition at present. Clinically, pemphigus is characterized by painful and non-healing ulcerations that rupture soon after forming in the oral cavity (6). Histopathology, a valuable examination showing intra-epidermal cleft for the diagnosis and differential diagnosis of bullous diseases, is considered the ‘gold standard’ diagnostic method by researchers (7). However, the epithelium is very susceptible to damage during biopsy and sample preparation, which can affect the diagnosis (5). Direct immunofluorescence (DIF), showing ‘fishnet appearance’ in pemphigus and also considered as the ‘gold standard’ in some research, also inevitably increases patients’ oral pain when it is performed...
in clinic (8). Indirect immunofluorescence (IIF) showing intercellular deposition of IgG is a reliable method for the diagnosis of pemphigus, which could detect the immunoglobulin G (IgG) autoantibodies against keratinocyte cell surfaces (9). While enzyme-linked immunosorbent assay (ELISA) is a commercial method, which detects the circulating antibodies against recombinant desmoglein antigens, the anti-Dsg 1 and anti-Dsg 3 ELISA also provide objective, quantitative, and reproducible data (10). Studies support the use of ELISA and IIF as complementary tests for the serological diagnosis of pemphigus (11). In addition, although the Tzanck smear test is a simple, rapid, and reliable cytological technique, only a few studies have analyzed its diagnostic value (12).

This study attempted to analyze and compare the diagnostic value of the Tzanck smear test, IIF, and ELISA to improve the diagnosis and treatment of pemphigus in dental clinics.

**Materials and methods**

Thirty-three patients diagnosed as pemphigus at the Department of Oral Medicine, Peking University and Hospital of Stomatology, from January 2010 to August 2014 were included in this research. They were having all the types of pemphigus vulgaris, and no treatment history before the test was done. Patients with pemphigoid, discoid lupus erythematosus, or erosive lichen planus were selected as controls over the same period. General information about patients, including their name, gender, age, address, and the clinical features, including size/number/location of the oral mucosa and skin lesions, was recorded. Clinical examination, histopathological examination, and immunological examination were performed for each patient. Pemphigus was diagnosed if patients fulfilled both the clinical and histological criteria: (i) the presence of chronic erosions, blistering, and exudation of oral mucosa, with or without blistering or erosions of skin or other mucosal junctions and (ii) the histopathological findings of intra-epidermal blisters with acantholysis [Ikeda et al. (13) and Kershenovich et al. (14)].

Histopathology was performed on the standard hematoxylin–eosin-stained sections. Criteria for the diagnosis of pemphigus included intra-epidermal blisters and suprabasilar acantholysis.

Tzanck smear tests of the lesions were performed for patients with pemphigus and control subjects. Acantholytic cells with a deep-dyed large nucleolus indicated pemphigus.

Indirect immunofluorescence (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) assays were performed via a standard technique using monkey esophagus as the substrate; the presence of intercellular IgG supported the diagnosis of pemphigus.

Desmoglein-1 and desmoglein-3 (Medical & Biological Laboratories CO., LTD, Aichi, Japan) were detected using the commercially available ELISA tests, and an index value higher than 20.0 IU/ml (15) indicated a positive reaction.

All Tzanck smear tests, IIF, and ELISA samples were checked by two trained examiners (Kappa value: 0.8).

This retrospective study was approved by the Ethics Committee of Peking University School of Stomatology.

The statistical analyses, including the sensitivities and specificities of the different tests, and the serial test were calculated as follows:

\[
\text{Sensitivity} \quad (%) = \frac{\text{True positive}}{\text{True positive} + \text{False negative}} \times 100.
\]

\[
\text{Specificity} \quad (%) = \frac{\text{True negative}}{\text{True negative} + \text{False positive}} \times 100.
\]

Serial test for A, B, and C (A, B, and C represent different tests):

\[
\text{Sensitivity} \quad (A + B + C) \quad (%) = \text{Sensitivity} \quad (A) \times \text{Sensitivity} \quad (B) \times \text{Sensitivity} \quad (C)
\]

\[
\text{Specificity} \quad (A + B + C) \quad (%) = \text{Specificity} \quad (A + B) + [1 - \text{Specificity} \quad (A + B)] \times \text{Specificity} \quad (C)
\]

\[
\text{Specificity} \quad (A + B) \quad = \text{Specificity} \quad (A) + [1 - \text{Specificity} \quad (A) \times \text{Specificity} \quad (B)]
\]

**Results**

Data from 33 patients with pemphigus (22 females, 11 males, mean age: 50.00 ± 10.40 years) were analyzed and compared with those from 61 controls (38 females, 23 males, mean age: 58.50 ± 12.70 years). The results of each test are presented in Tables 1–3. The sensitivity and specificity of the Tzanck smear test were 96.7% and 60%, respectively, while the sensitivities and specificities of IIF.

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**Table 1** Results of the Tzanck smear test

<table>
<thead>
<tr>
<th>Results of the Tzanck smear test</th>
<th>'Golden criteria'</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pemphigus</td>
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<tr>
<td>Positive</td>
<td>29</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
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</table>

**Table 2** Results of indirect immunofluorescence

<table>
<thead>
<tr>
<th>Results of indirect immunofluorescence</th>
<th>'Golden criteria'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pemphigus</td>
</tr>
<tr>
<td>Positive</td>
<td>28</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
</tr>
</tbody>
</table>

**Table 3** Results of enzyme-linked immunosorbent assay

<table>
<thead>
<tr>
<th>Results of enzyme-linked immunosorbent assay</th>
<th>'Golden criteria'</th>
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<tr>
<td></td>
<td>Pemphigus</td>
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<tr>
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<td>28</td>
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<td>Negative</td>
<td>5</td>
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</table>
The sensitivities of the serial test for the Tzanck smear test and IIF and the serial test for the Tzanck smear test and ELISA were both 82.0%, but the specificity of the serial test for the Tzanck smear test and ELISA was much higher (98.7% vs. 96.7%). The specificity of the serial test for IIF and ELISA was 99.7%, whereas the sensitivity was much lower (71.9%). The sensitivity and specificity of the serial test for the Tzanck smear test, ELISA, and IIF were 69.5% and 99.9%, respectively (Table 5).

All data are summarized in Tables 1 and 5. Based on these data, we formulated a diagnostic algorithm for pemphigus, as illustrated in Fig. 1.

Table 4  Sensitivity, specificity, and positive and negative predictive value for each assay

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tzanck smear tests</td>
<td>96.7 (95% CI 80.9–99.8%)</td>
<td>60 (95% CI 46.5–72.2%)</td>
<td>54.7 (95% CI 40.6–68.2%)</td>
<td>97.3 (95% CI 84.2–99.9%)</td>
</tr>
<tr>
<td>IIF</td>
<td>84.8 (95% CI 67.3–94.3%)</td>
<td>91.8 (95% CI 81.2–96.9%)</td>
<td>84.8 (95% CI 67.3–94.3%)</td>
<td>91.8 (95% CI 81.2–96.9%)</td>
</tr>
<tr>
<td>ELISA</td>
<td>84.8 (95% CI 67.3–94.3%)</td>
<td>96.7 (95% CI 87.6–99.4%)</td>
<td>93.3 (95% CI 76.5–98.9%)</td>
<td>92.2 (95% CI 82.0–97.1%)</td>
</tr>
</tbody>
</table>

Table 5  Sensitivity and specificity for serial tests

<table>
<thead>
<tr>
<th>Serial Tests</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tzanck smear tests + IIF</td>
<td>82.0</td>
<td>96.7</td>
</tr>
<tr>
<td>Tzanck smear tests + ELISA</td>
<td>82.0</td>
<td>98.7</td>
</tr>
<tr>
<td>IIF + ELISA</td>
<td>71.9</td>
<td>99.7</td>
</tr>
<tr>
<td>Tzanck smear tests + ELISA + IIF</td>
<td>69.5</td>
<td>99.9</td>
</tr>
</tbody>
</table>

IIF, indirect immunofluorescence; ELISA, enzyme-linked immunosorbent assay.

and ELISA were 84.8%, 84.8%, 91.8%, and 96.7%, respectively (Table 4).

The sensitivities of the serial test for the Tzanck smear test and IIF and the serial test for the Tzanck smear test and ELISA were both 82.0%, but the specificity of the serial test for the Tzanck smear test and ELISA was much higher (98.7% vs. 96.7%). The specificity of the serial test for IIF and ELISA was 99.7%, whereas the sensitivity was much lower (71.9%). The sensitivity and specificity of the serial test for the Tzanck smear test, ELISA, and IIF were 69.5% and 99.9%, respectively (Table 5).

All data are summarized in Tables 1 and 5. Based on these data, we formulated a diagnostic algorithm for pemphigus, as illustrated in Fig. 1.

Discussion

Pemphigus is a rare group of autoimmune bullous diseases characterized by the production of antibodies against desmoglein-1 and/or desmoglein-3 (1), although DIF, IIF, ELISA, the Tzanck smear test, and histopathology can be used to diagnose the disease.

In the present study, the clinical data of 33 patients with pemphigus and 61 controls were retrospectively analyzed. The sensitivities and specificities of the Tzanck smear tests and ELISA were 96.7%, 84.8%, 60%, and 96.7%, respectively (Table 4), which are consistent with the results of some previous studies. Durdu et al. (12) reported a sensitivity and specificity of the Tzanck smear test of 100% and 43.3%, respectively. Zagorodniuk et al. (9) demonstrated that the sensitivity and specificity of ELISA for pemphigus vulgaris were 81% and 94%, respectively. However, our results of IIF differed somewhat with those of some of previous studies. In the present study, the sensitivity and specificity of IIF were 84.8% and 91.8%, respectively. Using the monkey esophagus as the substrate for pemphigus vulgaris, Zagorodniuk et al. (9) demonstrated that the sensitivity and specificity of IIF were 81% and 100%, respectively. The different conclusion may be attributed to the racial difference or the fact that this study did not consider subtypes of pemphigus, and all subtypes were calculated together.

Studies support the use of ELISA and IIF as complementary tests for the serological diagnosis of pemphigus, and it has also been shown that the titer of serum antibodies correlates with the activity and severity of the disease (11). In the present study, the sensitivities of ELISA and IIF were both 84.8%, but the specificity of ELISA was higher than...
that of IIF (96.7% vs. 91.8%). The results were consistent with those of some previous studies. Kulkollakarn et al. (16) demonstrated that the specificity of ELISA using recombinant human desmoglein-1 and desmoglein-3 was higher than that of IIF in the detection of serum IgG antibodies in patients with pemphigus.

Although IIF, ELISA, and the Tzanck smear test can be used to diagnose the disease, each of them somehow has some drawbacks that would affect the diagnosis. IIF is a reliable method for the diagnosis of pemphigus which could detect the autoantibodies, but the overall sensitivity of this test is dependent on the type of substrate used (17). Although ELISA is a commercial method and can provide objective, quantitative, and reproducible data, it detects circulating antibodies only, and no cytological message presented (10). Tzanck smear test relies on the pathogenetic mechanism of acantholysis (18). In this process, the coherence between epidermal cells is lost due to breakdown of their intercellular bridges (19). The cells remain intact, but are no longer attached to each other; they tend to become rounded, resulting in intra-epidermal clefts, vesicles, and bullae (20). Tzanck preparation offers a more immediate answer than does serology, so it is a rapid and valuable cytodiagnostic technique to rule out pemphigus, which varies from the serological diagnostic techniques of IIF and ELISA and which is used to investigate the characteristics of individual patients. But only a few studies have analyzed its diagnostic value (12).

Pemphigus is a potentially life-threatening autoimmune bullous disease. Systemic corticosteroids are the first-line established therapy used to manage pemphigus (21). However, despite advances in management, severe complications, including a mortality rate estimated at 5–10%, preclude prolonged use (22). The oral presentation of the disease is often the first sign that can lead to the final diagnosis, it is very important for the dental practitioner to establish the diagnosis at an early stage to initiate further investigations and treatment. Therefore, more practical diagnostic strategy is very important for early and accurate diagnosis in dental clinics.

The sensitivities and specificities of various serial tests were compared in the present study. The sensitivities of the serial test for the Tzanck smear test and IIF and the serial test for the Tzanck smear test and ELISA were both 82.0%, but the specificity of combination of the Tzanck smear test and ELISA was much higher (98.7% vs. 96.7%). The specificities of the serial test for IIF and ELISA and of the serial test for the Tzanck smear test, ELISA, and IIF were much higher, at 99.7% and 99.9%, respectively, but their sensitivities were much lower, at 71.9% and 69.5%, respectively. Therefore, we proposed an algorithmic approach to the diagnosis of pemphigus, and the serial test for the Tzanck smear test and ELISA is recommended for use in dental clinics.

The limitations of our study were its retrospective nature. Firstly, the precise data about certain clinical feature such as disease severity were lacked which might affect ELISA results. Secondly, we diagnosed pemphigus based on histopathological findings. However, on some clinical situations taking a biopsy was difficult particularly in severe cases whose oral mucosa was severely destructed which might affect the results. What’s more, the number of the patients was insufficient. Larger studies are required to prove the diagnosis value of these tests for pemphigus in future.

In conclusion, according to the results of the present study, we proposed an algorithmic approach to the diagnosis of pemphigus, and the serial test for the Tzanck smear test and ELISA is recommended for common use in dental clinics.

References


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Hong Hua selected the topic, designed the study, and modified the article. Li Chunlei modified the article. Tingting Zhou and Siyue Fang selected and analyzed the data and wrote the article. This work was supported by Beijing Natural Science Foundation (ref. 7142168).

**Conflict of interest**

All authors claimed no conflict of interest.