Comparison of Immunofluorescence and Desmoglein Enzyme-linked Immunosorbent Assay in the Diagnosis of Pemphigus: A Prospective, Cross-sectional Study in a Tertiary Care Hospital

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Abstract

Background: Pemphigus is an acquired immunobullous disorder in which antibodies are directed against epidermal cadherins. Despite the commercial availability and less cost of enzyme-linked immunosorbent assays (ELISAs) to detect antidesmoglein 1 (Dsg1) and anti-Dsg3, immunofluorescence is still widely used for confirmation of diagnosis. Aims: (1) To compare the usefulness of indirect immunofluorescence (IIF) and ELISA tests in the diagnosis of pemphigus. (2) To find the clinical correlation between the tests and severity of the disease. Materials and Methods: Sixty-one patients (27 women and 34 men, age distribution from 20 to 75) were clinically diagnosed as pemphigus (pemphigus foliaceus - 11, pemphigus vulgaris - 50) and were recruited for the study. IIF and Dsg ELISA were performed and the findings were compared with each other and with the pemphigus area activity score. Data were entered in SPSS and were analyzed using Kruskal–Wallis test. Results: There was a moderate positive correlation between the cutaneous score and Dsg1 titer, and mucosal score and Dsg3 titer. The titer of IIF showed statistically significant positive correlation with the cutaneous score but not the mucosal score. Dsg ELISA showed higher sensitivity (90.2%) than IIF (75.4%) in the diagnosis of pemphigus. Conclusions: Dsg ELISA is a more sensitive method than IIF and shows more correlation with the disease severity.

Key Words: Desmoglein enzyme-linked immunosorbent assay, indirect immunofluorescence, pemphigus

Introduction

Pemphigus is an immunobullous disorder wherein antibodies (Abs) are directed against desmogleins (Dsgs) belonging to epidermal cadherin family and is classified into pemphigus vulgaris (PV) and pemphigus foliaceus (PF) with minor subtypes. The hallmark of pemphigus is the finding of auto-Abs against keratinocyte cell surfaces in vivo and in vitro detected by immunofluorescence, direct (DIF) and indirect (IIF). The cell staining pattern in DIF and IIF is almost identical with PV and PF. The major autoantigens for PV and PF have been identified to be Dsg3 and Dsg1, respectively. Cloning of these genes coding for the major pemphigus antigens has enabled the production of recombinant proteins, which enable commercially available enzyme-linked immunosorbent assays (ELISAs) to measure the level of Abs against Dsg1 and Dsg3.

Despite the commercial availability and cheaper cost of ELISAs to detect antidesmoglein 1 (Dsg1) and anti-Dsg3, immunofluorescence is still widely used for confirmation of diagnosis in most centres. So far, the studies comparing the two investigations have shown variable results, and we could not find any similar study reported in South India. Our aims and objectives were to compare the usefulness of IIF and ELISA tests in the diagnosis of pemphigus and to find the clinical correlation between the tests and severity of the disease.
Materials and Methods
A total of 61 patients, clinically diagnosed as pemphigus, above 18 years of age (PF - 11, PV - 50) were included in the study. Institutional ethical clearance was obtained (IEC 315/2012). A written informed consent was taken. A detailed history and clinical evaluation were done and clinical details were recorded in a standard pro forma. The cutaneous and mucosal pemphigus area’s activity score was calculated by the same person.[4]

A serum sample for IIF and Dsg1 and Dsg3 ELISA was collected. Tzanck test, biopsy for DIF or histopathology, was done in selected cases. IIF was done according to the usual protocol,[5-10] and sections were mounted using buffered glycerol and observed under fluorescence microscope. Chicken wire pattern of immunofluorescence was looked for, and the intensity of staining was graded subjectively as strong positive (+++), moderately strong (++), weakly strong (+), and negative (−). The IIF was labeled as negative or positive in 1:10 titer or positive in both 1:10 and 1:100 titers, substrate being normal human skin. Dsg1 and Dsg3 ELISA were performed in accordance with the standard method [Figure 1].[11,12]

The values of Dsg Abs 1 and 3 were given in relative units (RU)/ml.
- 20 RU/ml was the cutoff
- <20 RU/ml: Negative
- >20 RU/ml: Positive.

Statistical analysis
Statistical analysis was done using SPSS version 16 (SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc). Dsg1 and Dsg3 values were compared with the cutaneous and mucosal scores, respectively, using correlation test. The mean cutaneous and mucosal scores were calculated separately for patients with negative IIF, patients with IIF positive in only 1:10 dilution, and for patients with IIF positive in both 1:10 and 1:100 titers.

Kruskal–Wallis test was done to see whether the difference in the mean cutaneous score of the above three classes was statistically significant.

Results
The mean age was 43.25 years ± 13.39, maximum patients being in the 3–50 age groups (30).

In maximum patients, the initial site was the oral cavity (27), followed by the trunk (11), scalp (11), face (3), upper (3), lower limbs (2), and neck (1).

All other demographic data and pemphigus area activity scores are shown in Tables 1–3.

Indirect immunofluorescence
IIF was positive in 46 patients. In 14 patients, IIF was positive at 1:10 titer only and the rest 32 patients had positive IIF at both 1:10 and 1:100 dilutions.

Desmoglein enzyme-linked immunosorbent assay and indirect immunofluorescence interrelation
Dsg ELISA was negative in 5 out of 61 patients. Of these five patients, IIF was positive in four patients. IIF was negative in 15 cases where Dsg was positive. Both were negative in one case.

Direct immunofluorescence
DIF was done in 43 patients and was positive in 42 with intercellular staining of IgG and C3 in the epidermis.

Sensitivity of desmoglein enzyme-linked immunosorbent assay and immunofluorescence with respect to clinical diagnosis
Considering the clinical diagnosis as a gold standard, the sensitivity of Dsg ELISA was 90.2%, IIF was 74.5%, and DIF was 97.67% [Table 4].

Specificity could not be done as controls were not taken.

Table 1: Gender and type distribution

|          | Men     | Women   | Total |
|----------|---------|---------|-------|
| PV       | 50 (82%)| 11 (18%)| 61    |
| PF       | 27 (44%)| 34 (56%)| 61    |

Table 2: Duration

|          | Range            | Mean          |
|----------|------------------|---------------|
| Disease  | 5 days-7 years   | 342.57±517.17 |
| Treatment| 0-72 months      | 13.3 months±18.18 |

Table 3: Pemphigus area activity score

|              | Range | Mode | Mean   |
|--------------|-------|------|--------|
| Mucosal score| 0-6   | 3    | 24.9±1.6 |
| Cutaneous score| 0-16.4| 0    | 2.5±3.2 |

Figure 1: Desmoglein enzyme-linked immunosorbent assay test result

Table 4

|          |          |          |
|----------|----------|----------|
|          | Sensitivity | Specificity |
| Dsg ELISA| 90.2%     |            |
| IIF      | 74.5%     |            |
| DIF      | 97.67%    |            |
Sensitivity of indirect immunofluorescence and desmoglein enzyme-linked immunosorbent assay with respect to direct immunofluorescence

Studies have shown DIF as a better diagnostic test for pemphigus than IIF. Considering DIF as a gold standard, the sensitivities of IIF and Dsg ELISA were calculated and found to be 95.2% for Dsg ELISA and to be 85.7% for IIF [Table 5 and 6].

Desmoglein value and pemphigus area score correlation

Correlation between Dsg1 and Dsg3 values and the cutaneous and mucosal scores, respectively, were done and graded as mild (correlation coefficient <0.3), moderate (0.3–0.7), and strong (correlation coefficient >0.7). There was moderate correlation between cutaneous score and Dsg1 levels (correlation coefficient 0.444, \( P < 0.0001 \)) and mucosal score and Dsg3 levels (correlation coefficient of 0.474, \( P = 0.000 \)).

Indirect immunofluorescence titer correlation with the mucosal and cutaneous scores

The mean cutaneous and mucosal scores were tabulated separately for patients with negative IIF (0), patients with IIF positive in only 1:10 dilution (1), and for patients with IIF positive in both 1:10 and 1:100 titers (2).

The mean mucosal score was 3 ± 1.6 in all three classes. Whereas, the mean cutaneous score was different: 0.4 ± 1.75 in negative IIF, 0.7 ± 1.98 in IIF positive only in 1:10 dilution, and 2.9 ± 3.5 in IIF positive in both 1:10 and 1:100 titers [Table 7].

Kruskal–Wallis statistical test showed statistically significant difference (\( P = 0.001 \)). Thus, there was a statistical significant increase in titer of positivity of IIF with the increase in cutaneous but not the mucosal score.

Correlation of the titers of desmoglein enzyme-linked immunosorbent assay with duration of disease and treatment

There was no significant correlation between the duration of the disease, treatment, and the Dsg values.

Table 4: Sensitivity of desmoglein ELISA and IIF with clinical diagnosis as gold standard

|                | Desmoglein | Positive | Total |
|----------------|------------|----------|-------|
| **Negative**   |            |          |       |
| IIF (1:10 dilution) |      |          |       |
| Positive       | 5          | 41       | 46    |
| Negative       | 1          | 14       | 15    |
| **Total**      | 6          | 55       | 61    |

Desmoglein sensitivity - 90.2%

Discussion

Our study evaluated 61 patients, 34 men and 27 women, with a peak age of onset in the third to fourth decades of life. Twenty-eight patients (45.9%) had both cutaneous and mucosal involvement, 16 patients (26.23%) had only cutaneous involvement, and 14 patients (23%) had only mucosal involvement.

Mucosal cells exhibit more of Dsg3 and skin cells, Dsg1. Patients with mucosal predominant PV have sera which are negative against Dsg1 but positive for Dsg3 Abs and patients with PF are positive for Dsg1 and negative for Dsg3 Abs. Patients with mucocutaneous involvement were positive for both Dsg1 and Dsg3. Therefore, the clinical phenotype of pemphigus is defined by its auto-Ab profile.

A study done in India with 44 patients showed that skin and oral lesions correlate with Dsg1 and Dsg3 Abs, respectively. Dsg3 levels were higher in patients with extensive oral mucosal involvement but did not always correlate with disease severity.

There was a direct relationship between the severity of skin involvement and the levels of the Dsg1 Abs with skin involvement.

A study done in Taiwan with 143 patients showed that Dsg1 and Dsg3 ELISAs are highly sensitive and specific tests and can serve as a useful adjunct tool for the initial diagnosis of pemphigus. However, it did not show a correlation between values and clinical scores. Another study done in Beijing showed that Dsg3 ELISA values “fluctuate in parallel with disease activity and are useful to monitor disease activity, predict flares or relapses, and plan the schedules for tapering the drugs.”
In our study, there was a moderate (0.3–0.7) correlation between both Dsg3 titers and the mucosal score (0.444) and Dsg1 titer and the cutaneous score (0.474) [Figure 3]. Most of the patients had a Dsg titer of ≥200 RU/ml. The mean cutaneous scores for Dsg3 titers of <200 RU/ml and ≥200 RU/ml were calculated. Similarly, mean mucosal scores for Dsg1 titers of <200 RU/ml and ≥200 RU/ml were calculated.

The disease was significantly more severe in patients with higher titers (≥200 RU/ml) of Dsg based on statistics. There was no correlation between the Dsg titers and the duration of the disease or duration of treatment. Moreover, Dsg ELISA had high sensitivity in diagnosing patients with pemphigus (90.2%). The sensitivity considering DIF to be the gold standard was 95.2%.

Figure 2: Flow chart
Ravi, et al.: Comparison of IIF and desmoglein ELISA in diagnosis of pemphigus

ELISA was more cost-effective method compared to IIF. Studies have shown the higher accuracy of the ELISA technique over IIF in measuring the pemphigus Ab titers. IIF is used widely since the 1960s for monitoring disease activity, but IIF pemphigus Ab titers do not always correlate well with actual disease activity.

IIF is a semiquantitative technique and is time-consuming, and titers may not be available when therapeutic intervention is required. Dsg ELISA is quantitative. Therefore, the value of IIF is limited for monitoring pemphigus.

In a cross-sectional study of 61 PV patients done in Iran, there was a significant correlation between the cutaneous, mucosal scores, and the IIF titer, especially with the mucosal score, perhaps due to the usage of human prepuce as a substrate.

A study was done to compare five different epithelial substrates showed a statistically significant correlation between the IIF titer and the PV disease severity irrespective of the substrate. In PF, a positive correlation was obtained only with rabbit esophagus. In both PV and PF, there were individual patients in whom intercellular Ab titer was positive when there was no clinically evident disease, and some patients with extensive disease had IIF negative. Due to this, the use of IIF to monitor disease may not be justifiable.

In our study, the sensitivity of IIF taking clinical diagnosis as a gold standard in diagnosing pemphigus was found to be 74.5%. The sensitivity of IIF considering DIF to be the gold standard was 85.7%.

The absence of difference in the severity of the disease with an increase in titer of IIF in our study could be due to the use of human skin as a substrate.

The mean cutaneous score was different in the three classes being 0, 0.7, and 2.9 in patients with negative IIF, patients with IIF positive in only 1:10 dilution, and for patients with IIF positive in both 1:10 and 1:100 titers, respectively, with a statistical significant difference in mean.

Therefore, there is a significant positive correlation between the IIF titer and the cutaneous but not the mucosal score.

Many studies have found antigen-specific ELISA which has been shown to be more sensitive than immunofluorescence. ELISA using Dsg1 and Dsg3 offers the same specificity as IIF.

The titer of ELISA correlates better than IIF with disease activity.

Limitations of our study

Controls were not used in our study. Therefore, it was not possible to calculate the specificity of the tests.

Dsg titer calculation was done manually with the help of a graph sheet. A program in which only the optical density of the three calibrations, controls, and the patient serum value need to enter may provide more accurate results.
Mucosal substrates were not obtained for IIF which may have resulted in many of the patients with predominantly mucosal lesions to have negative IIF. Hence, studies with controls, mucosal substrates, and more number of patients in early pemphigus not started on treatment would be needed to further validate these findings.

Moreover, follow-up studies of patients to see the correlation of the scores with disease remission are needed. This would be helpful in deciding the point at which treatment can be tapered or stopped.

**Conclusions**

There is moderate positive correlation between the cutaneous score and Dsg1 titer and mucosal score and Dsg3 titer. Therefore, Dsg1 and Dsg3 titers can be used to assess the severity of pemphigus.

The titer of IIF shows statistically significant positive correlation with the cutaneous score but not the mucosal score. This could be due to the usage of skin and not mucosa as a substrate for IIF.

Dsg ELISA showed higher sensitivity (90.2%) than IIF (75.4%) in the diagnosis of pemphigus.

Dsg ELISA is a more cost-effective method than IIF and easier too in case of DIF, and if done with a large number of samples at one sitting, the cost-effectiveness could be increased due to the less number of microtiter wells used for the calibrators and the controls.

Dsg ELISA is a more sensitive and cost-effective method and could be used instead of immunofluorescence in assessing the disease severity.

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**Conflicts of interest**

There are no conflicts of interest.
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