Evaluation of the Role of Oral Mucosal Direct Immunofluorescence and Salivary Desmoglein 1 and 3 Enzyme-Linked Immunosorbent Assay in Patients With Oral Mucosal Pemphigus

Abstract

Background: Pemphigus vulgaris (PV) is characterized by antibodies against desmosomal adhesion proteins desmoglein (Dsg) 1 and 3 which can be detected by direct immunofluorescence (DIF) or enzyme-linked immunosorbent assay (ELISA). Oral lesions usually precede cutaneous lesions and an early diagnosis can prevent mortality and morbidity. Dsg antibodies can be detected by ELISA in saliva of patients with oral mucosal pemphigus. This study compares oral mucosal DIF with the salivary Dsg1 and 3 ELISA. Materials and Methods: A total of 26 biopsy and/or DIF-proven PV patients with oral erosions without cutaneous lesions were included in the study. Biopsy of oral mucosa was taken for DIF by standard method. Saliva sample was obtained and processed for ELISA. The results were then compared. Results: Out of 26 patients, 22 (84.6%) had a positive oral mucosal DIF and four patients (15.4%) had negative DIF. Nine patients (34%) had positive salivary Dsg3 ELISA. Seven patients (27%) had positive salivary Dsg1 ELISA. Taking oral DIF as the gold standard, the sensitivity of salivary Dsg1 ELISA was 31.8% and of salivary Dsg3 ELISA was 40.9%. Conclusion: Although DIF is the gold standard for the diagnosis of PV, salivary Dsg1 and 3 ELISA can also be used in the diagnosis of oral pemphigus.

Keywords: Oral DIF, oral mucosal pemphigus, salivary desmoglein ELISA

Introduction

Pemphigus vulgaris (PV) is a chronic autoimmune vesiculobullous disorder which manifests with severe painful oral erosions and flaccid fluid filled blisters over the skin and/or mucosa with antibodies directed against desmoglein (Dsg) 1 and/or 3. The gold standard test used for diagnosis of PV is direct immunofluorescence (DIF) of peri-lesional skin or mucosa which demonstrates intercellular deposits of IgG and/or C3 in a fishnet pattern. Other tests that aid in the diagnosis include indirect immunofluorescence and Dsg enzyme-linked immunosorbent assay (ELISA).

The disease usually begins with only oral lesions which may precede the cutaneous lesions by many months or years. Early identification and treatment of the disease helps in preventing severe mortality and morbidity.

Saliva has been evaluated as a potential diagnostic biological tool in the diagnosis of autoimmune diseases.[1,2] It consists of cytokines and hormones which could be transferred through the capillary walls in the salivary glands. Detection of anti-Dsg antibodies by ELISA in the saliva has been used in the diagnosis of PV and can be a simple, non-invasive, and alternative method to DIF. The purpose of our study is to evaluate the role of DIF and salivary Dsg 1 and 3 ELISA in patients with oral mucosal pemphigus.

Materials and Methods

The present study was a cross-sectional study conducted from January 2020 to July 2021 in the department of dermatology, pathology, and microbiology in a tertiary care hospital. Institutional ethics committee approval was obtained before starting the study (project no. 19/336). Informed and written consent were taken from all patients prior to the study. Twenty-six patients diagnosed as oral mucosal pemphigus either by histopathology.
and/or DIF were enrolled in the study. The duration of disease was less than 3 months in all the patients with no history of any previous treatment. Biopsy from oral mucosa was obtained for DIF, and an intercellular staining pattern with IgG and/or C3 was diagnostic of PV. Salivary samples were collected by the following method: The patients were explained to collect unstimulated whole saliva in their oral cavity for 5 minutes without swallowing and then instructed to spit it into sterile plastic containers. The undiluted saliva samples were then centrifuged at 3700 g for 10 minutes, and the supernatant saliva was separated for further evaluation to detect anti-Dsg1 and Dsg3 antibodies by ELISA. A titre value >13.4 RU/ml was taken as a positive result for salivary Dsg3 and a titre value >7.7 RU/ml was taken as a positive result for salivary Dsg1 ELISA. The procedure was performed using the EUROIMMUN (Medizinische Labordiagnostika AG, Germany) kit.

**Results**

Out of 26 patients, 22 of them showed positive oral mucosal DIF results. The four patients who showed negative results for DIF had histopathological features suggestive of PV.

Thirteen patients (50%) showed positivity to either Dsg1 or Dsg3 salivary ELISA, out of which three patients showed positivity to both Dsg1 and Dsg3.

Seven patients (27%) had positive results for salivary Dsg1 ELISA.

Nine patients (34%) had positive results for salivary Dsg3 ELISA.

The four patients who were negative for oral DIF showed negative results with salivary Dsg ELISA also.

Taking oral DIF as the gold standard, the sensitivity and specificity were assessed for salivary Dsg ELISA. Salivary Dsg1 ELISA showed a sensitivity of 31.8% and a specificity of 100% [Table 1], whereas salivary Dsg3 ELISA showed a sensitivity of 40.9% and a specificity of 100% [Table 2].

| **Table 1:** Comparison of salivary Dsg 1 ELISA and oral DIF |
|-----------------|------------------|
| **SALIVARY DESMOGLEN 1 ELISA** | **ORAL DIF** |
| POSITIVE | 7 | 0 |
| NEGATIVE | 15 | 4 |
| **SENSITIVITY-31.8%, SPECIFICITY-100%** |

| **Table 2:** Comparison of salivary Dsg 3 ELISA and oral DIF |
|-----------------|------------------|
| **SALIVARY DESMOGLEN 3 ELISA** | **ORAL DIF** |
| POSITIVE | 9 | 0 |
| NEGATIVE | 13 | 4 |
| **SENSITIVITY-40.9%, SPECIFICITY-100%** |

**Discussion**

PV manifests as an autoimmune bullous disorder with a chronic course, characterized by autoantibodies against desmosomal proteins such as Dsg1 or 3. In majority of the cases, the first clinical manifestation of the disease is seen to occur in the oral mucosal surfaces and may remain confined to the oral mucosa for a long period of time.

DIF of skin or oral mucosa is currently the gold standard test for the detection of PV in which intercellular deposits of IgG and/or C3 are demonstrated.

Saliva has been previously reported and studied extensively for its role as a biological tool to identify a number of autoimmune conditions. Although secretory IgA is the significant immunoglobulin detected in saliva, IgG is also found in saliva, the source of which is from the serum and hence, reflects the barrier integrity of mucosal epithelium.

Similarly, it has been found that saliva also contains the anti-Dsg antibodies and many studies have demonstrated that salivary Dsg ELISA can be used for the diagnosis of PV with high accuracy.

Mortazavi et al. compared the salivary anti-Dsg1 and 3 with serum ELISA anti-Dsg1 and 3 for diagnostic accuracy. In this study comprising 86 patients, the serum anti-Dsg1 and 3 ELISA were positive in 72.1% and 96.5% patients, respectively, and the salivary anti-Dsg1 and 3 ELISA were positive in 36% and 73% patients, respectively.

Hallaji et al. suggested that higher serum anti-Dsg1 in patients with severe cutaneous and mucosal lesions may be transmitted paracellularly via the disrupted oral mucosa resulting in higher salivary anti-Dsg1.

In a study by Maryam Koopai et al., serum and salivary Dsg ELISA results were correlated. Dsg1 and Dsg3 ELISA in serum showed sensitivities of 82% and 80%, respectively. Dsg3 and Dsg1 salivary ELISA showed sensitivities of 50% and 46%, respectively.

Andreadis et al. reported sensitivities of salivary Dsg 1 and Dsg 3 to be 45% and 93% accordingly. It was reported that the titres of anti-Dsg1 and 3 antibodies detected in both serum and saliva of PV patients demonstrated a statistically significant correlation, implying that saliva could be used as a biological tool for disease diagnosis and severity monitoring, and also for the early identification of relapses.

Dipankar De et al. reported a study comprising 43 patients with PV with predominant mucosal involvement. Salivary and serum Dsg1 and 3 levels demonstrated a statistically significant correlation. Salivary Dsg1 and 3 showed sensitivities of 90.7% and 88.4%, respectively.

S Ali et al. conducted the study with 23 patients with PV and detected salivary anti-IgG to Dsg 3 in 14/23 (61%) patients and serum anti-IgG antibodies in 17/23 (74%) patients with a strong positive correlation.
However, till date, there is no study comparing oral DIF and salivary Dsg ELISA in patients with oral mucosal pemphigus. In our study, using oral DIF as the gold standard, the following results were assessed; the sensitivity of salivary Dsg 1 and Dsg 3 ELISA was found to be 31.8% and 40.9%, respectively. Although DIF of the skin or mucosa is said to be the gold standard for diagnosis of PV patients, the use of saliva as an alternative in diagnosis has several advantages. Salivary Dsg ELISA is very simple to perform and is non-invasive. It is difficult to diagnose oral mucosal pemphigus patients without cutaneous lesions and is cumbersome to obtain oral biopsy sample for DIF. Salivary Dsg ELISA can be used as an alternative method in such patients. However, studies with a larger sample size are required.

**Conclusion**

Although DIF is the gold standard for the diagnosis of PV, anti-Dsg antibodies can be detected in the saliva of patients with oral mucosal pemphigus without cutaneous lesions and can be used as an alternative method in the diagnosis.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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