Sensitivity of direct immunofluorescence in oral diseases. Study of 125 cases

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Abstract

Direct immunofluorescence (DIF) is widely used for the diagnosis of bullous diseases and other autoimmune pathologies such as oral lichen planus. There is no evidence in the literature on how the following variants influence the detection rate of DIF: intraoral site chosen for the biopsy, perilesional locus or distant site from the clinical lesion, number of biopsies and instrument used.

Objectives: to determine if the following variants influenced the sensitivity (detection rate): intraoral site chosen for the biopsy, perilesional or distant site from the clinical lesion, number of biopsies and instrument used (punch or scalpel).

Material and methods: A retrospective study was done at the Cátedra de Patología y Clínica Bucodental II at the Facultad de Odontología, Universidad de Buenos Aires; 136 clinical medical histories were revised for the period March 2000 – March 2005 corresponding to patients with clinical diagnosis of OLP and bullous diseases (vulgar pemphigus, bullous pemphigoid and cicatricial pemphigoid).

Results: DIF detection rate was 65.8% in patients with OLP, 66.7% in cicatricial pemphigoid patients, in bullous pemphigoid 55.6%, in pemphigus vulgaris 100%, and in those cases in which certain diagnosis could not be obtained, the DIF positivity rate was 45.5% (Pearson $chi^2(4)= 21.5398$ Pr= 0.000). There was no statistically significant difference between the different sites of biopsy (Fisher exact test: 0.825). DIF detection rate in perilesional biopsies was 66.1% and in those distant from the site of clinical lesion was 64.7% (Pearson chi^2 (1)= 0.0073 Pr= 0.932). When the number of biopsies were incremented, DIF detection rate also incremented (Pearson $chi^2= 8.7247$ Pr= 0.003). The biopsies taken with punch had a higher detection rate than those taken with scalpel (39.1% versus 71.7%) (Pearson $chi^2= 49.0522$ Pr= 0.000).

Conclusion: While not statistically significant, the tendency outlined in this study indicates there are intraoral regions in which the detection rate of the DIF technique is higher than others: mouth floor, hard palate, superior labial muco-

sa, ventral face of tongue. This finding could allow a choice of accessible locations and easy operator manipulation, even in distant places from the clinical lesion. Perilesional biopsies have a detection rate similar to those taken distant from the clinical lesion, and those taken with punch have a higher sensitivity rate than those taken with scalpel (both differences were statistically significant).

Key words: *Direct immunofluorescence, oral lichen planus, pemphigus vulgaris, cicatricial pemphigoid, biopsy, sensitivity.*

Introduction

The accurate diagnosis of bullous and other immune diseases of the skin requires a clinical evaluation, histologic and immunofluorescence findings.

Direct immunofluorescence (DIF) use in diagnosis of bullous diseases and other immune diseases, such as oral lichen planus, has been of great value in confirming diagnoses, especially those in subepidermal bullous diseases that often have overlap in clinical and histological findings.

Direct immunofluorescence (DIF) detects immunoglobulins and complement components within biopsy specimens of patient's tissue. For bullous diseases, DIF is performed using perilesional skin or mucosal tissue; for lichen planus, vasculitis and connective tissue diseases, lesional skin or mucosa is needed. DIF has been used as a diagnostic tool for approximately four decades (1- 6).

Its use has helped the understanding of the physiopathology of some bullous diseases, making possible gnosologic replacements and new classifications (7-10).

Biopsy of lesional tissues for DIF is problematic since immune deposits are degraded by intense inflammation or damage in the basal membrane zone, rendering the DIF falsely negative. Currently, DIF is therefore performed in perilesional tissues (2).

Despite the wide use of this tool, little is known about the best anatomic site for taking the sample, especially when diseases affect multiple oral regions.

The objective of this study was to determine whether DIF sensitivity (detection rate) was influenced by the following variables: oral region chosen for the biopsy, sample taken perilesional or from any site away from the clinical lesion, number of biopsies taken, and whether a punch or a scalpel was used.

Material and Methods

We reviewed 136 clinical medical histories of patients who attended our clinic (Cátedra de Clínica y Patología Bucodental II, Facultad de Odontología, Universidad de Buenos Aires) with oral lichen planus or bullous diseases (pemphigus vulgaris, bullous pemphigoid or cicatricial pemphigoid), from March 2000 to March 2005.

Inclusion criteria were: bullous diseases and oral lichen planus patients of any age and gender, who had a histopathology study and DIF performed. Patients without any of these diseases were excluded. If the site of the biopsy for the DIF was not specified in the chart, or there was some other concomitant diagnosis such as squamous cell carcinoma, candidiasis or leukoplakia, patients were also excluded. In 11 cases, histopathology, DIF or clinical diagnosis were not concordant, so those charts were also excluded; leaving 125 cases for statistical analysis. In 11 cases of bullous diseases, an accurate diagnosis was not possible (non defined cases), for the DIF was negative in 6 cases, and didn't match the histology in 5 other cases.

The clinical relevant data taken into account were: age, gender, biopsy site, previous medication, histological diagnosis and its correlation with the suspected disease (oral lichen planus, vulgar pemphigus, bullous pemphigoid, and cicatricial pemphigoid).

We considered perilesional biopsies those taken within a radius of 1 cm of the clinical lesion, and distant biopsies were those taken outside this radius.

We also considered whether the biopsy was split in two for both studies (histology and DIF) or if two samples were taken separately for each study. The number of biopsies taken for DIF was also taken into account.

All biopsies were taken under similar circumstances and were analyzed in the same laboratory.

For hematoxylin–eosin staining, samples were first fixed in 10% formaldehyde and then stained properly; for DIF, samples were embedded in a gaze with saline solution, placed in a sterile flask, kept at 3-5 ºC and immediately transported to the pathology department, where they were processed.

For the DIF technique, 2 micron sections were cut in a cryostat at -20ºC and then placed on special slides. The sections were then fixed 20 min. in acetone, air-dried and afterwards incubated for 24 hours at room temperature in 1:15 anti-serum. Slides were then washed thrice; three minutes each with a phosphate buffer solution, and mounted afterwards in glycerol-phosphate (1:1) solution. The coverslips were sealed with enamel, allowing conservation at 3-5ºC until diagnosis.

We considered a positive pattern for vulgar pemphigus when Ig M, IgG, IgA or C3 in the intercellular space was deposited around the keratinocyte cell surface resulting in a honeycomb pattern (11).

For subepithelial blistering diseases, IgM, IgG, IgA or C3 deposition along the basal membrane zone in a continuous lineal pattern was considered positive, as for lichen planus the fibrinogen deposition along the basal membrane zone was a continuous shaggy pattern (12) (Figure 1-3).

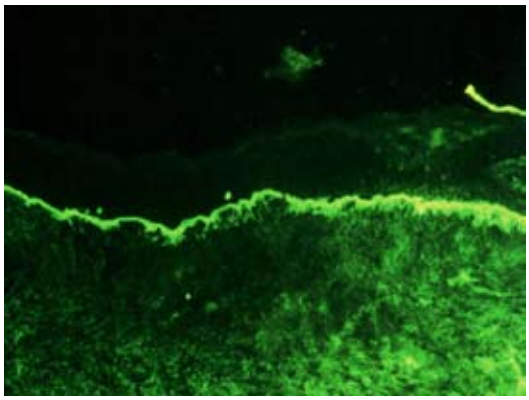

Fig. 1. DIF of lichen planus.

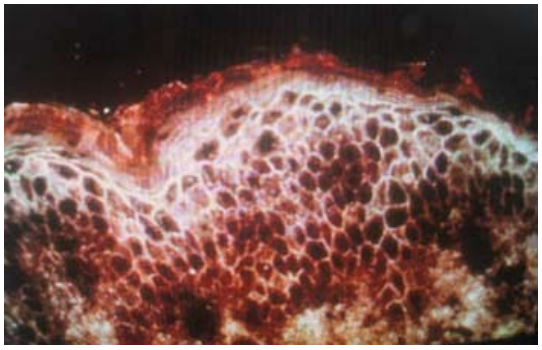

Fig. 2. DIF of pemphigus vulgaris.

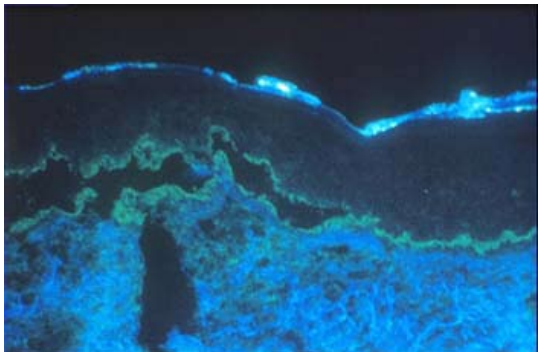

Fig. 3. DIF of bullous pemphigoid.

Differentiation between bullous pemphigoid and cicatricial pemphigoid was made on clinical grounds, since they both share histological and DIF findings and we don't perform immunoblotting in our clinic (13).

The study was conducted according to the principles of the Helsinki Declaration and its later amendments; and in accordance with the International Guidelines for Biomedical Investigation in Epidemiological Studies of the Council for International Organizations of Medical Sciences (CIOMS) and the Habeas Data Law (Protection Law of Personal Data).

Method detection rate was calculated as the total positive cases over the total occurred cases, and its percentage frequency was expressed regarding type of diagnosis.

In order to determine any association among the analyzed categorical variables a Chi square test was performed (14). For those cases in which the cross tabs had a cell with an expected frequency less than 5, a Fisher's exact test was computed. Any association was considered significant when the probability level was less than 0.05.

Results

129 DIF were considered, as some patients had more than one DIF performed.

Of the 125 patients, 32 were men and 93 were women. The range of ages was 11 to 95 years.

DIF detection rate was 65.8% in patients with oral lichen planus, 66.7% in patients with cicatricial pemphigoid, 55.6% in patients with bullous pemphigoid, and 100% in patients with vulgar pemphigus. In those cases with non-defined certainty diagnosis, DIF positivity was 45.5%. As seen in Table 1, there was a significant difference in frequency distribution regarding disease type. (Pearson chi^2 (4L)= 21.5 Pr= 0.000).

Table 1. DIF detection rate in different pathologies.

Pathology	Negative DIF	Positive DIF	Positivity rate
Oral Lichen Planus	27	52	65,80%
Cicatricial Pemphigoid	7	14	66,70%
Bullous Pemphigoid	4	5	55,60%
Vulgar Pemphigus	0	9	100%
Non-defined	6	5	45,50%

Pearson chi2 (4)= 21,5398 Pr=0.000; DIF: direct immunofluorescence.

Non-statistically significant differences were observed in the frequency of DIF positivity regarding different oral sites of biopsy sampling. (Fisher exact test: 0.825) (see table 2)

Table 2. IFD detection rate depending on biopsy site.

Site	Negative DIF	Positive DIF	Sensitivity
Inferior vestibular gingiva	3	3	3/6
Dorsal side of tongue	6	10	10/16
Superior vestibular gingiva	4	7	7/11
Inferior labial mucosa	2	5	5/7
Superior labial mucosa	1	3	3/4
Ventral side of tongue	1	5	5/6
Alveolar ridge mucosa	1	0	0
Buccal mucosa	22	48	48/70
Hard palate	1	4	4/5
Skin	0	2	2
Mouth floor	0	1	1

Fisher exact test= Pr 0.825.

DIF positive detection rate in perilesional samples was 66.1%, whereas in those samples taken in distant sites was 64.7%. (Pearson chi^2 (GL1) = 0.0073 Pr=0.932)
In table 3 we found that detection rate increased significantly with increased number of biopsies taken. (Pearson chi^2 (1GLf)= 8.72 Pr= 0.003).

Table 3. IFD detection rate depending on number of biopsies.

No. of biopsies	Negative DIF	Positive DIF	Detection rate
0,5	9	6	40%
1	21	56	72,70%
2	14	22	61,10%
4	0	1	100%

Pearson chi2 (1)= 8.7247 Pr=0.003
DIF: direct immunofluorescence

When punch or scalpel biopsies were compared, we observed that punch samples had a DIF positive detection rate statistically greater, being 71.7% as compared to 39.1% of those taken with scalpel. (Pearson chi^2 (1GL)= 49.05 Pr=0.000)

Discussion

As we can see in Table1, DIF sensitivity depends on the analyzed disease, being approximately 66% in OLP and cicatricial pemphigoid, and 100% in vulgar pemphigus, our results being in coincidence with the world's literature data (15-17).
As shown in Table 2, oral sites with greater DIF sensitivity were in decreasing order, mouth floor, the ventral side of the tongue, superior labial mucosa, hard palate and buccal mucosa. Oral sites with the least sensitivity were gingiva and dorsal side of the tongue. These differences were not statistically significant, partially due to the low power of the sample. Nevertheless, even though we can not speak of a statistically significant difference, we can point out a tendency (100% sensitivity in mouth floor or hard palate versus 30% in gingiva).
Making a correct diagnosis in a gingival sample can be a technical challenge, and might also leave a periodontal defect (18).
Some authors affirm that the epithelium is frequently detached from the chorion underneath or is lost during the sample processing, which leads to an incorrect sample preparation or to a wrong interpretation (4).
Other authors have proposed that gingival sampling has a low detection rate for the high degree of unspecific inflammation present in that area (13).
In this sense, we have observed as have other authors, that DIF biopsies taken from gingival tissue in general, and in several diseases in particular, have a detection rate lower than that of other localizations.
Even though it is recommended in the literature that DIF biopsies be taken perilesional, our study indicated the possibility for greater latitude in tissue sampling. When DIF sensitivity was analyzed, there was no significant difference between biopsies taken perilesional or distant from the clinical lesion.
We know that immune deposit can be present in the whole oral tissue, and not only in those localizations near the clinical lesions. It is also known, for instance, that even in ocular cicatricial pemphigoid cases without clinical oral lesions, biopsies for DIF might be taken from oral sites, for the immune deposit is also there, and therefore ocular morbidity can be avoided (12).
Presence of immune deposits in the whole oral tissue and not only in damaged tissue, might explain our finding that DIF was highly positive when biopsies had been taken from non-perilesional sites. This gives more options for tissue sampling that might prove useful, especially in cases when taking one perilesionally is technically difficult.
From results shown in Table 3, we conclude that splitting a biopsy for both hematoxylin-eosin and DIF is not convenient, for DIF sensitivity in these cases was significantly lower than the rest (40%).
In general, we conclude that there are some anatomic oral regions in which the DIF technique sensitivity is greater: mouth floor, hard palate, superior labial mucosa, and ventral side of the tongue. Knowing this may allow the physician to choose accessible and manipulable regions, even those that are not close to the clinical lesions.
We can also conclude from this work that punch samples have a greater detection rate for DIF than those taken with a scalpel.
The results shown in this study need confirmation by a randomized, controlled prospective study in order to validate the tendencies outlined here. This will substantiate the usefulness of this approach for improving the diagnosis of bullous diseases and OLP using DIF.

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