Dermatology Practical & Conceptual

Can Immunofluorescence on Skin/Mucosal Scraping Smear for Pemphigus Diagnosis Substitute Direct Immunofluorescence on Skin Biopsy?

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ABSTRACT

Introduction: Few studies have been conducted on the use of Direct Immunofluorescence (DIF) on skin/mucosal scraping smear for diagnosis of pemphigus disease; however, the diagnostic value of DIF on the smear has not been fully evaluated.

Objectives: The present study was carried out to assess the sensitivity and specificity of DIF on skin/mucosal smear for diagnose of pemphigus in the patients presenting with mucocutaneous erosive lesions.

Methodology: A total of 89 patients including 40 males and 49 females aged between 23 and 80 years old with various bullous disorders were enrolled in the study. For definite diagnosis, all the patients were subjected to lesional biopsy for pathological studies and perilesional biopsy for DIF studies. In all the cases, skin/mucosal scraping smears were prepared from the perilesional healthy skin/mucosa and were stained with immunofluorescence conjugated anti-IgG.

Results: Of 89 patients, 56 (63%) patients were diagnosed with pemphigus. Immunodeposits favoring the pemphigus were demonstrated in the 46 smears of 56 cases of pemphigus (sensitivity of 82%). No case with other types of bullous disease had positive DIF on the smear (specificity of 100%).

Conclusion: The findings of the study showed that the sensitivity of DIF on the smear is not high enough to allow us replacing the conventional DIF with smear-DIF for diagnosis of pemphigus, while the specificity of 100% would allow the unequivocal identification of a subset of patients with pemphigus.

Introduction

Pemphigus as a rare autoimmune blistering disease is characterized by the widespread flaccid blisters and erosions on the skin and mucous membranes [1]. The hallmark of pemphigus is finding the immunoglobulin G (IgG) autoantibodies against the cell surface of keratinocytes. Pemphigus is classified into subtypes based on the main autoantigen involved in the pathogenesis of disease [1]. Detection of IgG autoantibodies raised against the cell surface of keratinocytes is considered as the gold standard for diagnosis of pemphigus. Pemphigus can be differentiated from other vesiculobullous or pustular diseases through detection of these autoantibodies [2]. Direct Immunofluorescence (DIF) examination is the most reliable and sensitive diagnostic test used for all forms of pemphigus. DIF is able to show the IgG and C3 deposition around the epithelial cells, confirming the diagnosis of pemphigus [3].

Durdu et al have demonstrated that the keratinocyte cells obtained by Tzanck smear can be used as a substrate for DIF studies [4]. Obtaining the Tzanck smears is less invasive than skin or mucosal biopsy and would be useful in the pemphigus patients with conjunctiva involvement or inaccessible oral lesions that cannot be biopsied easily [5]. In this technique, preparation of the samples is much more rapid than the conventional DIF and there is no need for specialized equipment such as the cryostat.

Objectives

The current study was performed to investigate the usefulness of DIF on skin scraping smears obtained from intact perilesional skin in the patients with bullous/erosive disorders in order to evaluate the use of skin/mucosal smears as an alternative to skin or mucosal biopsies for diagnosis of pemphigus.

Methods

Patients

The study protocol was approved by the Institutional Ethics Committee and an informed written consent was obtained from all the patients.

A total of 89 consecutive patients with erosive, vesicular, bullous or pustular skin, or mucosal lesions were included in this study. Demographic, clinical and laboratory data including patients age, gender, and lesion location were recorded using a questionnaire. For definite diagnosis, all the patients were subjected to lesional biopsy for pathological studies and perilesional biopsy for DIF studies. In all the cases, smears were prepared from the perilesional healthy skin/mucosa and were stained with immunofluorescence conjugated anti-IgG.

Preparation of the Smears and Application of DIF on the Samples

For preparing the smears, the perilesional skin or mucosa adjacent to the fresh blister or erosion was first anesthetized through the intradermal lidocaine injection and then, they were gently scraped using the small curette. Then, the obtained cellular materials were spread as a thin layer onto at least two glass slides and were air-dried. The prepared smears were sent to the Department of Pathology for staining. Smears were incubated with fluorescein isothiocyanate (FITC)-conjugated goat antihuman IgG (CEDARLANE, lot number: 7201111401) for 30 minutes in a moist chamber at 37degree temperature. The sections were then washed in Phosphate-buffered saline (PBS) (2washes of 15 min each), mounted in buffered glycerol, and examined under fluorescent microscope.

Detection of the ring-shaped deposition of IgG on the individual acantholytic cells or the net-like intercellular fluorescence pattern when sheet of cells were present was considered positive for diagnosis of pemphigus (Figure 1).

The immunofluorescence (IF)-stained samples were studied independently by 2 of the authors. They were unaware of the results of the conventional DIF.

Calculation of the Diagnostic Value of IF -Stained Skin/Mucosal Scraping Smears

The parameters including sensitivity (ie the percentage of patients with positive conventional DIF whose IF-stained smear was positive), specificity (ie the percentage of patients with negative conventional DIF whose IF-stained smear was negative), Positive Predictive Value (PPV) (ie the percentage of patients with positive IF-stained smear whose conventional DIF was also positive) ,and Negative Predictive Value (NPV) (ie, the percentage of patients with negative IF-stained smear whose conventional DIF was also negative) were calculated to determine the diagnostic value of the IF-stained smear (Table 1). Kappa coefficient was calculated to evaluate the concordance of the IF-stained smear and conventional DIF, and the P value of less than 0.05 was considered as statistically significant.

Results

Totally, 89 patients (40 males and 49 females) aged between 23- 80 years were included in this study. Table1 shows the characteristics of the patients and their diagnosis based on the histopathological and conventional DIF results. Classical suprabasal acantholysis at the lesional biopsy and immune

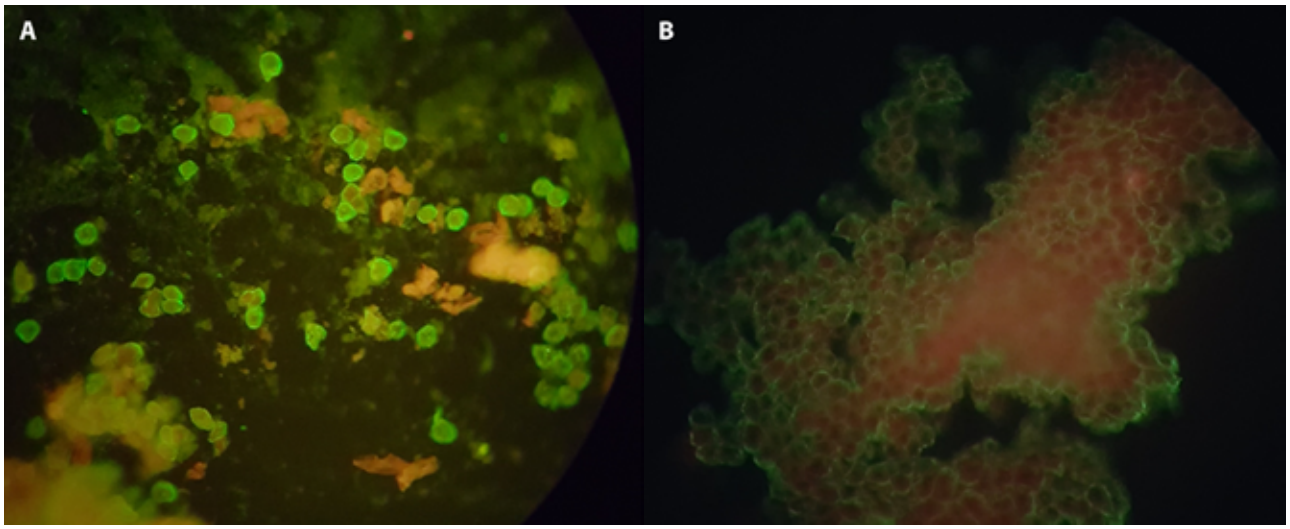

Figure 1. Positive direct immunofluorescence examination of skin scraping smear in a patient with pemphigus vulgaris shows immunoglobin G deposition around the individual acantholytic cells (A) and those in groups (B) (400×).

Table 1. Characteristics of Patients with Bullous/Erosive Lesion

Age, years Mean ±SD, range	46.60±12.16, (23-80)			
Gender, N(%)	Female	49 (55%)		
	Male	40 (45%)		
Diagnosis	Diagnose of patient with bullous disease based on histopathology of lesional samples, N		Intraepidermal Positive DIF on perilesional punch biopsy sample N(%)	Positive DIF on perilesional scraping smear N(%)
	Pemphigus	56	56 (100%)	46 (82%)
	Vulgaris	54	54	44
	Folliaceous	2	2	2
	Bullous pemphigoid	6	0(0%)	0(0%)
	TEN	3	0(0%)	0(0%)
	Erythema multiforme	3	0(0%)	0(0%)
	Fixed drug eruption	7	0(0%)	0(0%)
	Herpes zoster	3	0(0%)	0(0%)
	chicken pox	3	0(0%)	0(0%)
	Bullous impetigo	2	0(0%)	0(0%)
	Bite reaction	1	0(0%)	0(0%)
	Acute eczema	3	0(0%)	0(0%)
	Sweet syndrome	1	0(0%)	0(0%)
	Pustular psoriasis	1	0(0%)	0(0%)
Site of obtaining smear or biopsy for IF study N(%)				
	Oral mucosa	19 (21%)	16	12
	Extremities	9 (10%)	2	2
	trunk	40 (45%)	18	14
	Scalp	21 (24%)	20	18

DIF = Direct Immunofluorescence; IF = Immunofluorescence; SD = standard deviation; TEN = Toxic Epidermal; Necrolysis.

deposition compatible with the diagnosis of pemphigus (intercellular lattice-like pattern) were demonstrated in the IgG–stained perilesional biopsies of 56 cases.

IF on the smear was positive in 46 (82%) patients with pemphigus. IF on the smear had a sensitivity of 0.82 (95% Confidence Interval [CI] 0.72-0.92), a specificity of 1.00

Table 2. Distribution of Frequency of Dif on Tzank Smear and Conventional Dif on Skin/Mucosa Biopsy in Patients with Bullous/Erosive Lesions.

	Result of conventional DIF (golden criteria for pemphigus diagnosis)		
Results of DIF on Tzank smear	**Positive, N(%)**	**Negative, N(%)**	**Total, N(%)**
Positive, N(%)	46 (82%)	0 (0%)	46(55%)
Negative, N(%)	10 (18%)	33 (100%)	43(45%)
Total N(%)	56 (100%)	33 (100%)	89 (100%)

Kappa = 0.773 P < 0.001
DIF = Direct Immunofluorescence.

(95% CI 1.00-1.00), a PPV of 1.00 (95% CI 1.00-1.00), and a NPV of 0.77 (95% CI 0.64-0.89). A significant concordance was found between the results of IF-stained smears and those prepared by the conventional DIF for diagnose of pemphigus (Kappa= 0.773, P <0.001). Table 2 presents the data on IF-stained smears and those prepared by the conventional DIF.

Conclusions

Lesional skin/mucosal scraping called Tzanck smear is generally used for diagnosis of the Herpes Simplex Virus infections [6]. The presence of acantholytic cells accompanied by multinucleated giant cells is a characteristic cytological finding for diagnosis of herpetic infections [6]. This method has also been suggested as a simple and rapid technique to be used in diagnosis of pemphigus disease [6]. Cytological examination of the smears obtained from scraping of floor of the blisters in the pemphigus patients has shown the presence of typical acantholytic cells (or Tzanck cells). These cells are not pathognomonic for the pemphigus and are commonly observed in other types of bullous disease such as Hailey-Hailey disease and herpetic infections [4,6]. The cyto-diagnosis is not extensively used due to low specificity of this technique in diagnosis of the pemphigus. Positivity of acantholytic cells in the cases with pemphigus has been reported by 96.7%-100% while, the specificity of acantholytic cells for pemphigus has been reported by 43.3%-60% [4,7]. This means that, if we rely on the use of Tzanck smear alone, 40%-60% of the cases presented with erosive and bullous eruptions would falsely be diagnosed as pemphigus. Then, for definite diagnosis of pemphigus, autoantibodies raised against the epithelial cell membrane have to be detected by applying the DIF staining [3].

DIF analysis of the perilesional skin biopsy is the most accurate approach for diagnosis of pemphigus , showing IgG deposits on the surface of keratinocytes [3] . The direct immunofluorescence test on Tzanck smears has been proposed as a simple alternative to skin biopsy for diagnosis of pemphigus [4]. DIF examination of a Tzanck smear shows bright green fluorescence at the cell margins of single acantholytic cell or in the intercellular region in the case of cell clumps, compatible with positive IF pattern of pemphigus [4].

Although, the IF examination of skin scraping smear seems a simple and practical cytological technique for diagnosis of pemphigus, there is a limited evidence on the relative sensitivity and specificity of DIF on the smear compared to the DIF on skin biopsy as a gold standard.

According to the review of the literature, there are a few related studies with divergent results in this context. Durdu et al have reported about the typical IgG deposit around the acantholytic keratinocytes in the Tzanck smears of all (100%) the 20 patients with pemphigus [4]. Nonetheless, Aithal et al have shown that among 12 pemphigus patients with positive DIF on the skin biopsy, only 6 of them (50%) had positive DIF on the Tzanck smear [8].

In the current study, the result of DIF examination on the smear was positive in 46 (82%) of the pemphigus patients (out of 56 patients). Ten patients had negative results. The larger sample size or technical issue in the IF staining of the smears might explain observing these 10 false negative results.

It also could be attributed to the fact that the smears were taken from the healthy perilesional skin and not from the blister floor. In scraping of the intact skin, collected keratinocytes are mostly from the superficial epidermal layers where immune depositions are partly or completely absent in the subset of patients with pemphigus vulgaris. In pemphigus vulgaris, due to the difference in the relative amount of desmoglein 3 in the epidermal layers, occasionally the fluorescence may be limited to or more intense in the lower levels of the epidermis [2].

According to the results, the sensitivity and specificity of DIF on skin/mucosal smear for diagnosis of pemphigus were equal to 82% and 100%, respectively. This sensitivity was not high enough to allow us replacing the conventional DIF on skin biopsy with DIF on skin/mucosal smear, for diagnosis of pemphigus. In other words, approximately 20% of pemphigus patients would be missed if we rely on IF staining on the smear alone. Nonetheless, the observed specificity of 100% allows an extremely high level of confidence to diagnose the pemphigus in the case of positive DIF on the smear.

One limitation of this study is that the only DIF was used as a gold standard to differentiate pemphigus cases from other vesiculobullous diseases. Because of resource limitation anti desmoglein antibodies were not measured. Then we were unable to compare the diagnostic value of DIF on skin/mucosal smears with enzyme-linked immunosorbent assay for detecting anti-desmoglein 1 and 3.

Given that, DIF on the smear is a less invasive and much cheaper procedure compared to the DIF on biopsy, a plausible approach is that when a clinically suspicious pemphigus patient presents with the bullous lesions first, the DIF examination on the skin scraping smear is performed, and if it is positive then, the diagnose of pemphigus is confirmed while if, it is negative then, a biopsy must be taken for conventional DIF studies.

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