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Original article

Evaluation of sensitivity of Tzanck smear in pemphigus

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Abstract

Background Pemphigus is a group of immune-mediated, acantholytic disorders. Although direct immunofluorescence is the gold standard for the diagnosis of this group of diseases, it is not widely available and cost-effective in our country. Tzanck smear is a simple and easy test used to demonstrate acantholytic cells from the lesions of disease. The present study was planned to evaluate the sensitivity of Tzanck smear, in the diagnosis of pemphigus group of diseases.

Patients and methods Tzanck smears were prepared from 37 cases of active pemphigus (17 males and 20 females, age range 11-65 years), diagnosed by clinical, histopathological and direct immunofluorescence data. Patterns and positivity rate of Tzanck smears were recorded.

Results Overall sensitivity of Tzanck smears was 73%. Smears were positive in 75% of cutaneous lesions and 69% of mucosal lesions (p>0.05). Acantholytic cells were seen individually in all positive cases (100%), clumps (81%) and sheets (70%).

Conclusion Tzanck smears can be used as first-line investigation in our scenario. More sophisticated investigation e.g. direct immunofluorescence may be reserved for Tzanck–negative cases.

Key words
Tzanck smear, pemphigus, sensitivity

Introduction

Pemphigus is a group of immune-mediated, intraepithelial acantholytic blistering diseases, involving the skin and mucous membranes. Amongst the immunobullous disorders, it is the most common entity.[1] The disease is further divided into three types i.e. pemphigus vulgaris, pemphigus foliaceous, and paraneoplastic pemphigus, with regard to their distribution, morphological features, histopathologic and immunofluorescence findings, antigenic specifications, and associated conditions.[2] Direct immunofluorescence is an essential investigation for the diagnosis of all types of pemphigus with high degree of sensitivity and specificity.[1,2] However, the technique is expensive to install and maintain. Due to the same reasons this technology is available at only few centres in Pakistan.

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Tzanck, in 1947, pioneered the work on cutaneous cytology and evolved a method for the cytological diagnosis of various blistering diseases.[3] Tzanck smears are still widely used for the diagnosis of

acantholytic bullous diseases and herpes viral infections. This is a relatively simple and easy-to-perform technique. The present study was planned to evaluate the sensitivity of Tzanck smears in pemphigus in relation to the direct immunofluorescence, the gold standard investigation for this disease.

Patients and methods

This study was conducted at the Department of Dermatology, King Edward Medical College/Mayo Hospital, Lahore. Thirty seven cases of active pemphigus (17 males and 20 females, age range from 11 to 65 years), were included in the study. Clinical and investigative data were recorded on a specially designed pro forma. The diagnosis was confirmed by performing direct immunofluorescence on perilesional skin/mucosal biopsy specimens. Histopathology (with hematoxylin and eosin staining) on lesional skin and mucous membrane was performed to determine the subtype of pemphigus. Tzanck smears were prepared from cutaneous bullae (24 cases) or mucosal ulcers (13 cases) for Giemsa staining.

For Tzanck smears, a fresh, non-infected, unruptured bulla was selected. Lesions were wiped with alcohol and allowed to dry for one minute. After deroofing the blister, its floor was scraped with the edge of a scalpel blade. The material obtained was gently transferred from the blade to the glass slide to make a smear. In case of mucosal involvement similar smears were prepared from the floor of the erosions. For staining, the smears were flooded with diluted stain for 15 minutes. The excess stain was rinsed out and slides were allowed to dry. The stained smears were examined under light microscope for the presence of acantholytic cells.

For direct immunofluorescence study, perilesional skin/mucosal biopsies were wrapped in a gauze piece soaked with normal saline. These specimens were frozen and stored in liquid nitrogen immediately. For sectioning, the specimens were embedded in embedding medium, and cut at about -30ºC to a thickness of 5 microns and placed on a glass slide. Five sections per slide and 2-3 slides per patient were prepared. The sections were allowed to air-dry for about 10 minutes. For DIF staining of these biopsy sections, the slides along with positive and negative controls were rinsed in phosphate-buffered saline (PBS) (pH=7.2). The sections were overlaid with fluorescein isothiocyanate (FITC) conjugated antisera i.e. antihuman IgG, IgA, IgM, and C3 (The Anstar [Ltd], USA). The slides were incubated in a moist and closed plastic container for 20 minutes, followed by three washes of fifteen minutes each in PBS to remove the unreacted antiserum. They were allowed to drain, and the excess fluid was wiped off with dry cotton gauze. The slides were mounted using a drop of buffered glycerin as the mounting medium (90% glycerin in PBS). Finally, the specimens were examined under a fluorescence ultraviolet microscope for their immunofluorescence patterns.

Chi-square test was used for statistical evaluation.

Results

Of 37 cases, 31 (83.8%) had pemphigus vulgaris, 5 (13.5%) pemphigus foliaceus, and one (2.7%) pemphigus vegetans.

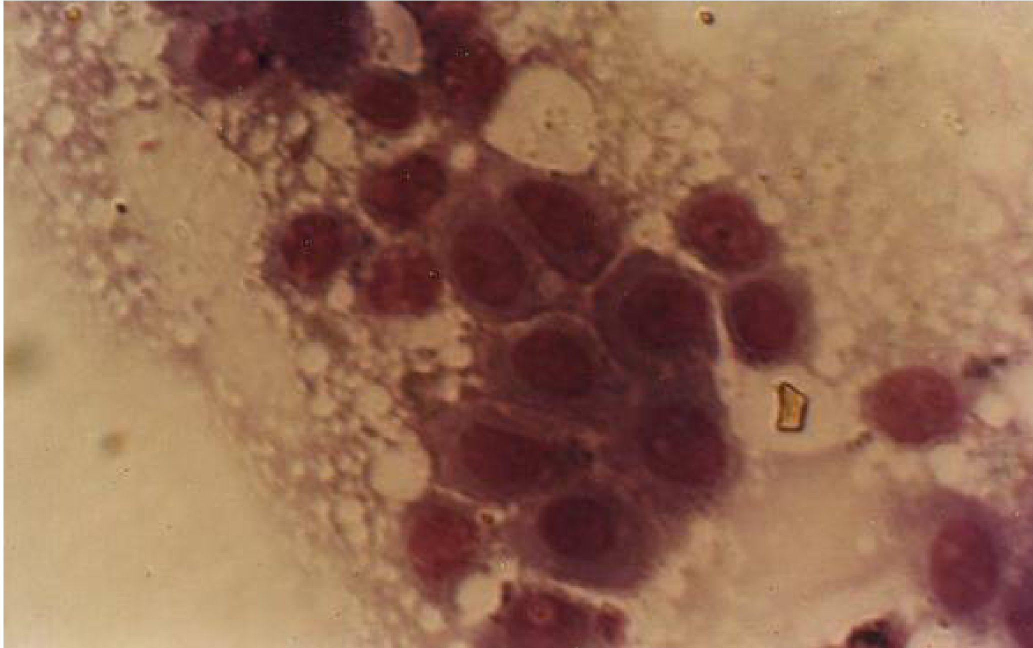

Figure 1 Tzanck smear with Giemsa staining showing a sheet and individual acantholytic cells.

Mean duration of the disease was 26 months ranging from three weeks to eight years.

Direct immunofluorescence showed deposition of immunoreactants at intercellular spaces (ICS) of epidermis in all (100%) patients. The most common immunoreactant detected was IgG, singly or in combinations, in all patients (100%) followed by C3 in 13 patients (35%), IgM in 2 patients (5.4%), and with IgA in one patient (2.7%).

On histopathological examination 15 patients were diagnosed as pemphigus vulgaris, 4 as pemphigus foliaceus and 1 as pemphigus vegetans. Histopathological features in 6 patients were nonspecific and their diagnosis was based on immunofluorescence findings and clinical features. Tzanck smears were prepared from cutaneous and mucosal lesions in 24 and 13 patients, respectively. Overall 27 patients (73%) showed positive results i.e. acantholytic cells, significantly lower as compared to DIF ($p<0.05$). The majority showed more than one morphological patterns. The cells in positive cases were individually scattered (100%) or in the form of clumps (81%) and sheets (70%) [**Figure 1**]. Positivity rate among the oral lesions was 69% as compared to 75% in skin lesions ($p<0.05$) [**Table 1**]. Morphological patterns in mucosal smears were identical to that of skin smears.

Discussion

Tzanck smears sensitivity was only 73% as compared to DIF, a less sensitive test but with certain advantages. Smears are easy to make and less traumatic as compared to skin or mucosal biopsies. There is no need to preserve the specimens in transport medium or to section them. By-passing these two important steps makes the test economical, time saving and technically less demanding. The technique of preparing the Tzanck smears has been established as an easy, early and

rapid aid to the diagnosis of pemphigus as described previously.[4,5]

Presently, there is a general agreement that direct immunofluorescence staining at ICS in epidermis is invariably positive in case of active pemphigus, the diagnosis of which should be seriously questioned if this is found negative.[2] However, this technique has certain limitations especially in countries with poor resources. It is expensive to install (cryocut, ultraviolet fluorescence microscope) and maintain e.g. fluorescein-labelled antisera, liquid nitrogen, different solutions etc. A special biopsy is required from the perilesional area, further traumatizing the patient. Cryostat sectioning, labelling, and interpretation of slides require much more expertise as compared to Tzanck smears. Tzanck smears, in our study, were negative in about one-fourth of cases. Different reasons for negative results may be the procedure limitations adopted for obtaining the cells on glass slides, inflammatory infiltrate destroying the antibody-labelled targeted cells, or partial treatment with glucocorticoids. In previous studies on the subject, Decherd *et al*.[6] performed the test on only two patients and reported positive results. Though the results were 100% but the total number of cases was very low.

Patterns of cells in Tzanck smears were in the form of individually scattered cells, clumps or sheets, identical from both oral and cutaneous lesions. This might be dependent on the severity of inflammatory reaction. Similarly, the positivity was similar in oral or cutaneous lesions (69% vs. 75%, $p>0.05$). No significant correlation of positivity of smears with age, sex or duration of the disease was detected.

Table 1 Sensitivity of Tzanck smear from cutaneous and oral lesions

	Skin lesions (n=24)	*Oral lesions* (n=13)
Positive	18 (75%)	9 (69%)
Negative	6 (25%)	4 (31%)

Concluding, though the Tzanck smear is less sensitive as compared to DIF on biopsy specimens, the simplicity of the test is a considerable edge. Keeping the sensitivity and simplicity together, the test may be used for screening pemphigus patients. Biopsy may be indicated in negative cases. The test may be especially helpful in situations where biopsy is refused or is difficult to perform. Collaboration of the test with clinical and histological parameters can further sharpen the diagnosis of pemphigus.

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