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Original research article

Utility of Tzanck smear cytology in diagnosis of vesiculo-bullous skin lesions: A Cross-sectional study

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Abstract

Introduction: Tzanck smear was first introduced in 1947 by Frenchman Tzanck as a tool of diagnostic cytology for vesiculobullous conditions, especially herpes simplex. With time it evolved and Tzanck smear findings for several other dermatological conditions like immunobullous disorders, genodermatosis, cutaneous infections, and cutaneous tumors have also been described. Tzanck smear is a rapid, inexpensive, simple, and sensitive outpatient cytology technique that can be performed with minimal patient discomfort and cost. Hence, we studied all the vesiculobullous lesions in our institute with special reference to immunofluorescence and its final clinical diagnosis.

Aims and Objective: This study aimed to highlight the diagnostic utility of Tzanck smear in vesiculobullous disorders in correlation with the clinical diagnosis.

Materials and Methods: A hospital-based cross-sectional study was carried out on all Tzanck smears received during twelve months (1/1/ 2017– 31/12/2017) after taking ethical committee clearance. The Tzanck smears were assessed under a microscope for specific findings and Statistical Package for the Social Science [SPSS] software was used to establish Tzanck smear utility in the diagnosis of vesiculobullous lesion.

Results: The present study included 68 cases. The majority of these Patients had viral infections (33) followed by immunobullous disorders (23). Among 33 Herpes viral cases, 20 showed multinucleated giant cells and 3 showed viral inclusions on cytology. Among 24 immunobullous lesions, 15 out 16 Pemphigus cases showed acantholytic cells, and the rest of all 8 bullous pemphigoid cases showed significant number of eosinophils. Histopathological correlation was available only for 24 out of 68 cases.

Conclusions: Tzanck smear test is a simple, easily applicable, rapid, and inexpensive test for the diagnosis of erosive vesiculobullous and can serve as a useful adjunct to routine histological examination.

Keywords: Multi Nucleated Giant Cell, Vesiculo-Bullous Lesions, Statistical Package for the Social Science

Introduction

Tzanck smear was first introduced in 1947 by Frenchman Tzanck as a tool of diagnostic cytology for vesiculobullous conditions, especially herpes simplex [1]. With time it has evolved further and entered into the diagnosis of several other dermatological conditions like immunobullous disorders, genodermatosis, cutaneous infections, and cutaneous tumors [1]. Tzanck smear is a rapid, inexpensive, simple, and sensitive outpatient cytology technique that can be performed with minimal patient discomfort and cost [2]. In some disease, cytological findings are diagnostic while in others they are only suggestive of disease and need to be confirmed by histopathology [3].

Our study is different from previous studies done as it includes all the vesiculobullous lesions and an extensive study was done with special reference to immunofluorescence in which diagnosis was not

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possible by histopathology. Our study and our institute stress the factors like proper clinical and diagnostic rapport or communication can help to alleviate patients from pain caused by biopsy. In our study we performed biopsy in only 24 cases rest were solved by just with cytology findings with proper clinical history, thus highlighting the importance of Tzanck smear.

Aims and Objective

This study aimed to highlight the diagnostic utility of Tzanck smear in vesiculobullous disorders in correlation with the clinical diagnosis.

Materials and Methods

This Hospital-based cross-sectional study was carried out on all Tzanck smears performed for twelve months (1/1/ 2017– 31/12/2017) in a tertiary care centre after taking ethical clearance from the committee. All consecutive patients with intact blisters or erosions, not on treatment, of all ages and both the gender groups, were included in the study. Patients taking treatment for the underlying vesiculobullous disorders within a period of two weeks prior were excluded from the study. Informed consent was obtained from all patients. Clinical and demographic details of all the patients were recorded.

Tzanck smear was obtained by gently scraping from the base of a fresh vesicle, blister, or pustule or directly from the crusted lesion. The youngest vesicle or bulla, preferably less than three days old, was preferred for sampling since older lesions may get crusted or secondarily infected and the characteristic cytomorphology may no longer be present.

Preparation of Tzanck smear

In clinically suspected viral infections samples were taken from a fresh vesicle, rather than a crusted one, to ensure maximum yield of viral infected cells. In other vesiculobullous lesions, the vesicle was unroofed, and the base of the lesion was gently scraped with a scalpel or the edge of a spatula. The material thus obtained was smeared onto two slides gently. Both air-dried and alcohol-fixed smears were prepared and subsequently stained with Leishman's and Papanicolaou stains.

The stained smears were subsequently examined under the light microscope for identification of viral cytopathic effect (multinucleated giant cells and viral inclusion), acantholytic cells, inflammatory cells, and any other cutaneous infections (fungal hyphae or bacterial colonies).

In Cases with discrepant histopathology diagnosis, cytology slides were reviewed for identifying additional features. Suggestive diagnosis of pemphigus was given after observing many acantholytic cells and typical Tzanck cells. Diagnosis of bullous pemphigoid was made if smears showed a significant number of eosinophils. In cases where smear showed multinucleated giant cells and viral inclusion diagnosis of viral infection was done.

Statistical analysis was performed using a statistical package for the social sciences software (Version 21.0; SPSS, Inc, Chicago, IL). The patient data for each demographic or histopathology characteristics were summarized as Mean ± standard deviation and incidence of characteristic within a particular group was calculated as percentage of entire study population. Fisher's exact test was performed and a p value of < 0.05 was considered statistically significant.

Results

The present study included a total of 68 patients with vesiculobullous skin lesions, who satisfied both inclusion and exclusion criteria. Most of the patients included were suspected viral infections (33), followed by autoimmune vesiculobullous disorders (16). Other dermatological skin lesion includes bullous pemphigoid (8), contact dermatitis (2), spongiform dermatitis (2), photoallergic dermatitis (1), Steven Johnson's syndrome (1), erythema multiforme (1), pyoderma (1), infective dermatitis (1), herpetiform dermatitis (1) and Tenia cruris (1). These diagnoses were made by correlating the clinical details and microbiology reports.

The majority group among the study population comprised of viral infection (33) with herpes zoster (18) and varicella (11) constituting the major group. The rest of the patients were herpes simplex (4). Most of the smears (20) among these revealed multinucleated giant cells. Intranuclear inclusions were seen in 4 out of 33 only.

Among the study group, 16 were Pemphigus, all except one revealed the presence of an abundance of acantholytic cells. Even though few other conditions like viral infection, spongiform dermatitis, and erythema multiforme show acantholytic cells, but they were only a few, only in Pemphigus disorder acantholytic cells were in plenty.

Among 8 cases of bullous pemphigoid, all of them showed the presence of epithelial cells and a significant number of eosinophils along with neutrophils. Few other cases like contact dermatitis and infected eczema showed eosinophils but not in significant number.

Other cases like 2 contact dermatitis, 1 erythema multiforme, 1 pyoderma, 1 infective dermatitis, 1 tenia

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cruris,1 photoallergic dermatitis, 2 spongiform dermatitis, and 1herpetiform dermatitis showed nonspecific neutrophilic and lymphocytes infiltrate.

Histopathological correlation was available for 24/68 patients. Direct immunofluorescence was done for 2 biopsies i.e., Pemphigus Vulgaris and bullous pemphigoid, and both correlated with the cytological and clinical diagnosis. Pemphigus Vulgaris showed IgG and C3 intercellular deposition and in bullous pemphigoid, it showed linear deposition near basement membrane.

Sensitivity and specificity which was calculated using a statistical package for the social sciences software (Version 21.0; SPSS, Inc, Chicago, IL) showed 60.6% sensitivity and 97.1% specificity with a significant p-value of 0.00001 in viral infection. In our study, there were significant numbers of eosinophilic infiltrate with a sensitivity of 100% and specificity of 95%. Also, the sensitivity and specificity of acantholytic cells in Tzanck smear in patients of Pemphigus was 93.7% and 96% with a significant p-value of 0.00001.

Table 1: Findings

Diseases	Findings
Immunobullous	
Pemphigus vulgaris	Acantholytic cells, hazy nucleoli
BP/SJS/Erosive LP	No acantholytic cells, plenty of leukocytes Eosinophils particularly in BP.
Toxic epidermal necrolysis	Necrotic basal cells, leucocytes, fibroblasts
Staphylococcal scalded skin syndrome	Dyskeratotic acantholytic cells, no/ little inflammation
Infective lesions	
Leishmaniasis	Leishman- Donovan bodies, Wright's cells
Herpes simplex / Varicella / Herpes zoster	Ballooning multinucleated giant cells
Molluscum contagiosum	Henderson- Patterson bodies
Genodermatosis	
Hailey- Hailey diseases	Acantholytic cells, normal nucleoli
Darier's Diseases	Corps ronds, grains
Table /Fig 1: Tzanck cytology findings in various dermatoses [3]	

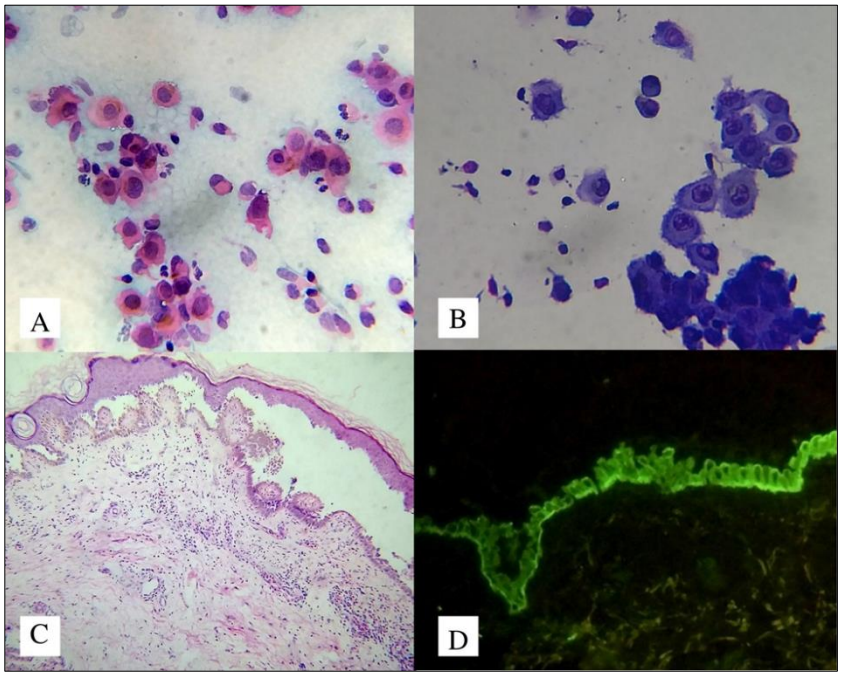

Fig 1: Pemphigus group showing acantholytic cells in A. Papanicolaou -40X and B. Leishman's -40X.C. Histopathology sections-10X showing Suprabasal bullae. D. Pemphigus vulgaris showed IgG and C3 intercellular deposition

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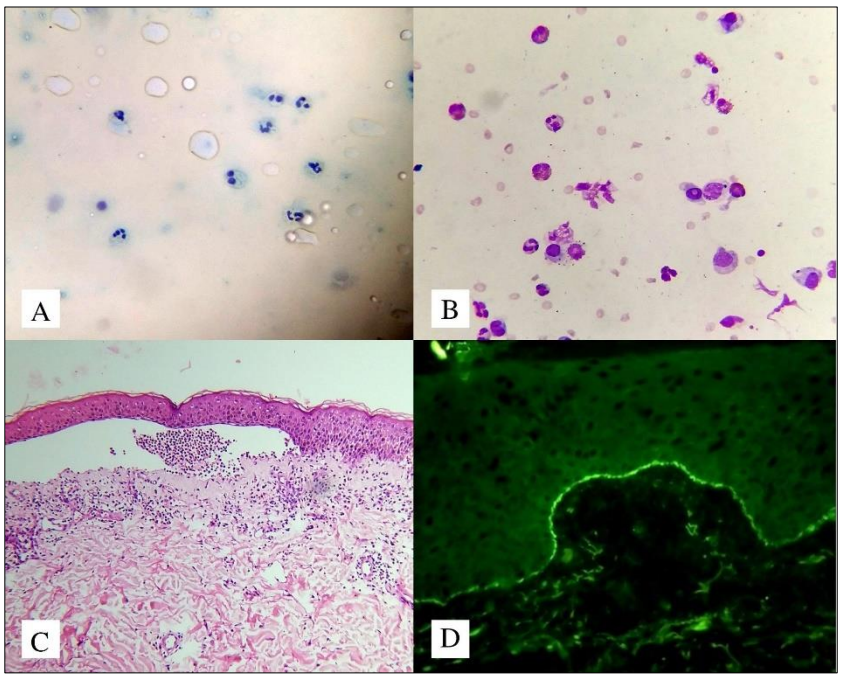

Fig 2: Bullous pemphigoid -significant number of eosinophils A. Papanicolaou -40X and B. Leishman's -40X.C. Histopathology sections-10X. D. Immunofluorescences showed linear IgG deposition near basement membrane

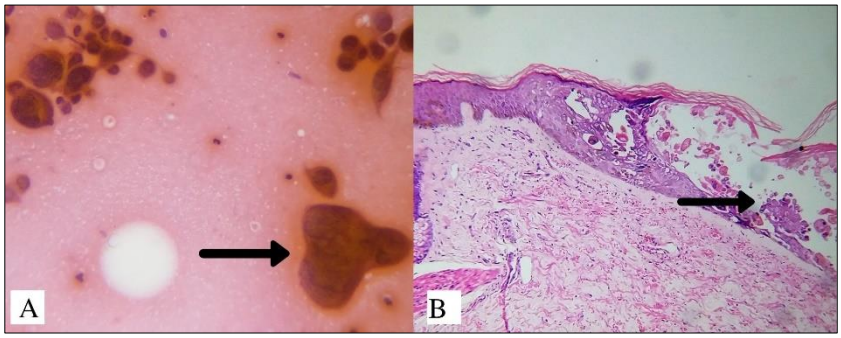

Fig 3: Herpes infection showing multinucleated giant cells A. Papanicolaou -40X and B. Histopathology sections-10X.

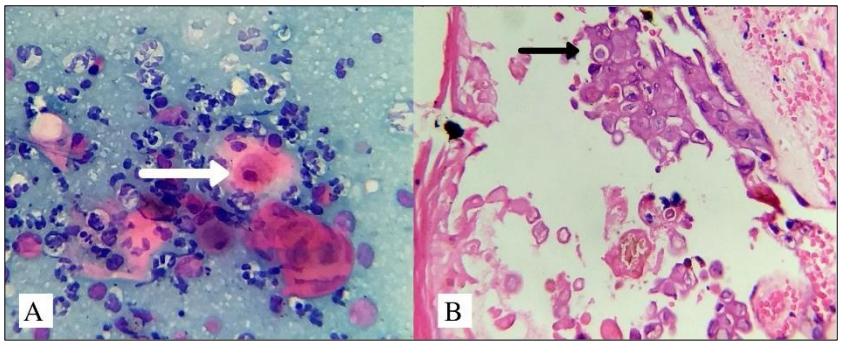

Fig 4: Herpes infection showing viral inclusion A. Papanicolaou -40X and B. Histopathology sections-10X.

Table 2: **Correlation between clinical diagnosis and Tzanck smears**

Correlation between clinical diagnosis and Tzanck smears	Fisher's exact test (P value)	Significance
Acantholytic cells and clinical diagnosis	0.00001	Significant
MNG cells and clinical diagnosis	0.00001	Significant
Eosinophils and clinical diagnosis	0.00001	Significant
Intranuclear inclusion and clinical diagnosis	0.1089	Not Significant
Table / Fig 6: Correlation between clinical diagnosis and Tzanck smear, applying Fisher's exact test		

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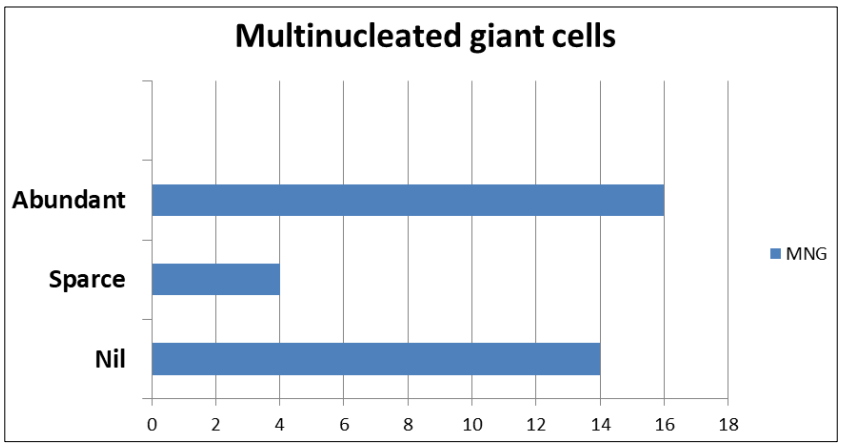

Fig 5: Distribution of Multinucleated giant cells in Tzanck smears of viral infection

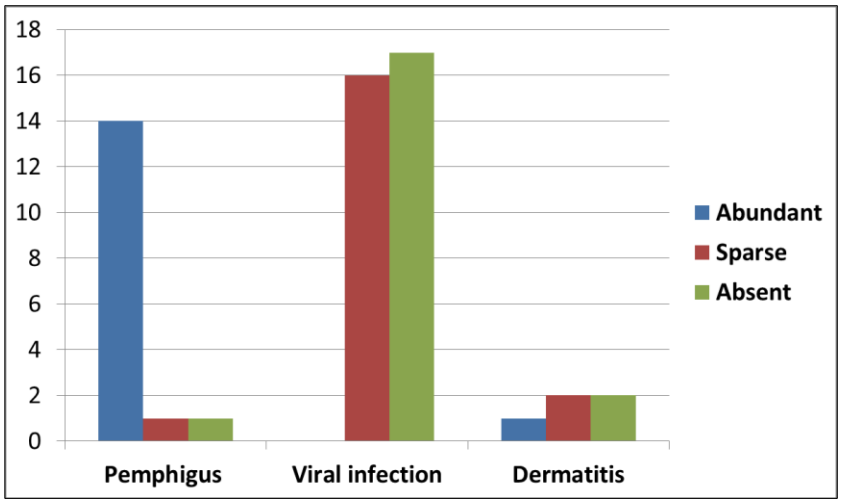

Fig 6: Distribution of acantholytic cells in Tzanck smears

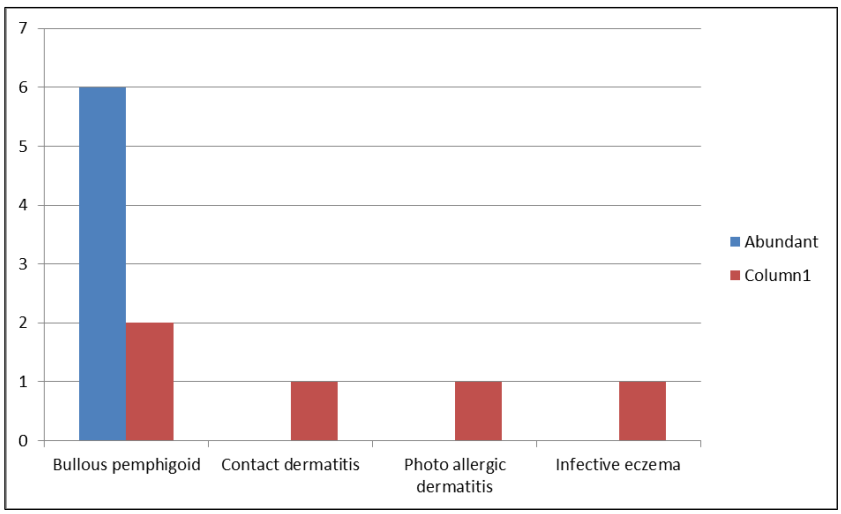

Fig 7: Distribution of eosinophils among Tzanck smears

Discussion

Among the 68 patients studied, 33 patients were clinically suspected viral infection which showed 60.6% sensitivity and 97.1% specificity with a significant p-value of 0.00001. These findings are comparable to Atiya *et al*, which showed sensitivity and specificity of 86.3% and 91.3% [4]. Durbu *et al*, also state sensitivity of 100%, 69.2%, and 59.7% for vesicular, pustular, and erosive skin lesions respectively. [5]. Inclusion bodies were identified only in 12% of cases stained by Leishman's and Papanicolaou stain which was not significant. This was similar to the Heera *et al*, study which showed inclusion in 18% of cases which

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is also not significant. [2]. According to Vincezo Roucco, Solomon A.R *et al*, the application of Tzanck smear helps less in differentiating varicella virus infection and herpes infections and this study also go in this support. [6, 7].

In our study sensitivity and specificity of acantholytic cells in Tzanck smear in patients of Pemphigus were 93.7% and 96% with a highly significant p-value of 0.00001. Hence Tzanck smear is particularly helpful in providing a provisional diagnosis of Pemphigus Vulgaris when the site of the lesion is not amenable for biopsy or the disease is in a very early stage. [3]. Similar results were reported by Heera *et al*, with 100% sensitivity and specificity [2]. Jameel *et al*, reported a sensitivity of 75% in the cutaneous lesion [8].

In our study, there were significant numbers of eosinophilic infiltrate with a sensitivity of 100% and specificity of 95%. Hence Tzanck smear is useful in differentiating the same from the pemphigus group. According to previous studies [3, 5, 6], Tzanck smear in the case of bullous pemphigoid was nonspecific which is not the case in our study and the study by Heera *et al*, hence making our study unique and different from earlier studies [2].

The utility of the Tzanck smear is no longer restricted to corroborate the diagnosis of pemphigus group of lesions and herpetic infections. It can even obviate the need for biopsy in certain cutaneous infections like Molluscum contagiosum, candidiasis, and leishmaniasis. The findings of the Tzanck smear should always be interpreted in an appropriate clinical context to optimally utilize the benefits of this old but valuable technique [1].

Typical acantholytic cells are usually observed on Tzanck smear in cases of Pemphigus Vulgaris, while other bullous lesions show scarcity of keratinocytes, absence of acantholytic cells, and relative predominance of inflammatory cells. Eosinophils are seen in abundance in cases of bullous pemphigoid.[3] Similar cytological features of pemphigus Vulgaris and bullous pemphigoid were also noted in our study. Some authors have even suggested the use of a direct immunofluorescence test for detection of immunoglobin deposits on Tzanck smear, to make the test more specific

The Tzanck smear findings are nonspecific in a vesiculobullous lesion of contact dermatitis, spongiform dermatitis, and photoallergic dermatitis [2].

Conclusion

Tzanck smear is a simple, inexpensive, rapid, and useful diagnostic tool. It does not require a specialized laboratory setup. Moreover, taking a Tzanck smear hardly causes any trauma or discomfort to the patient and therefore can be easily performed and repeated even in most timorous individuals, children, and difficult to biopsy sites such as lips, eyelids, or genitals. We recommend the use of the Tzanck smear as the first-line investigation for vesiculobullous, erosive, and pustular lesions for a rapid clinical diagnosis. It can serve as a useful adjunct to routine histological examination. Despite the development of many sophisticated techniques in the field of diagnostic methods, it carries its importance in the field of diagnostic cytopathology.

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