ORIGINAL RESEARCH PAPER

Pathology

EVALUATION OF SENSITIVITY AND SPECIFICITY OF TZANCK SMEAR COMPARED TO HISTOPATHOLOGY AND DIRECT IMMUNOFLUORESCENCE IN DIAGNOSIS OF IMMUNOBULLOUS DISORDERS

KEY WORDS: Pemphigus Vulgaris, Tzanck smear, DIF, sensitivity, specificity, PPV, NPV, accuracy

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ABSTRACT

Background: It has long been established that cutaneous cytology is helpful in the identification of immunobullous skin lesions. Tzanck smear test is a quick, easy, and affordable way to diagnose immunobullous skin lesions. The objective of the study was to evaluate the sensitivity and specificity of Tzanck smear compared to direct immunofluorescence and histopathology in diagnosis of immunobullous lesion. **Methodology:** This is a retrospective study conducted from January 2020- December 2022. Tzanck smear findings were assessed and correlated with histopathological diagnosis and direct immunofluorescence findings. The diagnostic value was compared with parameters like sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. **Results:** Total 52 patients were included with mean age of 52.46 ± 18.19 years and female-to-male ratio was 1.74. Majority of the patients were diagnosed with pemphigus vulgaris (51.92%). When compared to histopathological examination (HPE), Tzanck smear had sensitivity of 61.54%, PPV of 100%, and NPV of 0.00%. Tzanck smear had sensitivity of 63.27%, specificity of 66.67%, PPV of 96.87%, NPV of 10.00%, and diagnostic accuracy of 63.46% as compared to direct immunofluorescence (DIF). **Conclusion:** Tzanck smear is a quick and reliable tool for the evaluation of various immunobullous skin lesions. Additionally, it aids in the histological analysis of skin biopsies.

INTRODUCTION:

Immunobullous disorders are a class of autoimmune diseases that attack intracellular attachments within the epidermis and basement membrane zone, resulting in the development of cutaneous and mucosal blisters. The target antigens are parts of desmosomes or the adhesion complex, a functional component of the basement membrane zone.[1] If ignored, these immunobullous disorders may be related to considerable morbidity and mortality. Therefore, a precise diagnosis and therapy are required.[2]

The diagnosis of immunobullous disorders is based on classic signs and symptoms, conventional histology, and direct immunofluorescence (DIF).[3] DIF has been widely implemented in addition to the clinical and histologic features of vesiculobullous diseases and has emerged as an important diagnostic technique in the identification of immunobullous lesions of the skin.[4,5]

Arnault Tzanck, a Frenchman employed cytology for the first time in the diagnosis of pemphigus and herpes in 1947. Since then, the "Tzanck smear" method of cytology has been used to diagnose several vesiculobullous, erosive, tumoral and granulomatous conditions.[6] Tzanck smear is a simple, inexpensive, rapid bedside technique that can be utilised in the early diagnosis of immunobullous diseases, since histopathology and direct immunofluorescence, while specific, are time consuming and expensive.[1]

The sensitivity and specificity of Tzanck smear in diagnosing immunobullous diseases are varying from 70-100% as reported by different studies.[6,7,8] There is also paucity of data predicting the diagnostic value of Tzanck smear in immunobullous disorders in Indian population. Hence, we conducted this study with aim of evaluating the sensitivity and specificity of Tzanck smear compared to DIF and histopathology in diagnosis of immunobullous lesions.

MATERIALS AND METHOD:

This was a retrospective hospital record-based study. The study was conducted in the Department of Pathology, Yenepoya Medical College and Hospital, Mangalore. Patients' data from January 2020-December 2022 was retrieved from the records stored as digital form in the software (Backbone) from the Central Laboratory.

All cases presenting with dermatological lesions requiring cytological, DIF and histopathological evaluation were included in the study. Cases in which the material obtained were inadequate for interpretation and cases with inconclusive cytological diagnosis were excluded from the study.

To get sample for Tzanck smear for crusted ulcerative lesions, crusts were removed after soaking the affected area either with normal saline or in distilled water for 10 minutes and for nodular lesions, a small incision was made with a fine-edged scalpel blade. The cellular material from the area of incision was collected and then spread on to a clean glass slide to make a smear. The smear is fixed with methyl alcohol for 2-3 min and then stained with 2-3 drops of stock solution of May-Grunwald-Giemsa stain over a period of 5 to 10 minutes. The stock solution was prepared by diluting 1 part of stain with 3 parts of distilled water. The slide was washed quickly and allowed to dry and smear was finally examined under light microscope for cytological findings.

For Histopathological examination the samples were taken from post-operative excisional, incisional or punch biopsies and were fixed in 10% buffered formaldehyde, paraffin embedded and thin sections of around 4 microns were made and stained with hematoxylin and eosin stain for analysis of histological features.

Direct immunofluorescence reports were collected from the Department of Dermatology. Tzanck smear findings, DIF and histopathological diagnosis were analysed in all patients. The sampling was done using Simple random sampling technique.

Sample size was calculated based on the following formula:

$$\frac{Z^2_{1-\frac{\alpha}{2}} p(1-p)}{L^2} = \frac{1.96 \times 1.96 \times 0.16\,(1-0.16)}{(0.1)^2} = \frac{0.5161}{0.01} = 51.61$$

p = expected proportion = 16% = 0.16

L = Absolute precision required on either side of the proportion = 10% = 0.1

Z0.025 = 1.96 for 95% confidence interval.

Thus, sample size was calculated to be 51.61, rounded-off to 52 patients.

The incidence of diagnosis was expressed in frequency and proportion. To test the diagnostic accuracy of Tzanck smear with HPE and DIF, the parameters like sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were determined.

RESULTS:

A total 52 patients were included in this study. The age of the patients ranged from 13 to 92 years with a mean of 52.46 ± 18.19 years. Majority of the patients included in this study were females (63.46%) with a female-to-male ratio of 1.74. (Table 1)

Table 1: Demographic And Pathological Characteristics Of All Patients Included In The Study (N=52).

Characteristics		N (=52)	%
Age groups (years)	< 30	3	5.77
	30 – 60	33	63.46
	> 60	16	30.77
Gender	Male	19	36.54
	Female	33	63.46
Histopathologic findings	Pemphigus vulgaris	27	51.92
	Bullous pemphigoid	21	40.38
	Pemphigus foliaceous	4	7.69
Tzanck smear	Positive	32	61.54
	Negative	20	38.46
Direct immunofluorescence	Positive	49	94.23
	Negative	3	5.77

As shown in the histopathologic findings, majority of the patients were diagnosed with pemphigus vulgaris (51.92%), followed by 40.38% patients of bullous pemphigoid and 7.69% patients of pemphigus foliaceous. Tzanck smear were positive for acantholytic cells in 32 (61.54%) patients and 20 (38.46%) were negative for acantholytic cells. Out of 52, 49 (94.23%) were positive on direct immunofluorescence, while remaining were negative (5.77%).

Out of 27 patients with pemphigus vulgaris, 26 (96.29%) had positive findings on Tzanck smear, histopathology, and DIF (intercellular staining of epidermis with IgG and C3).

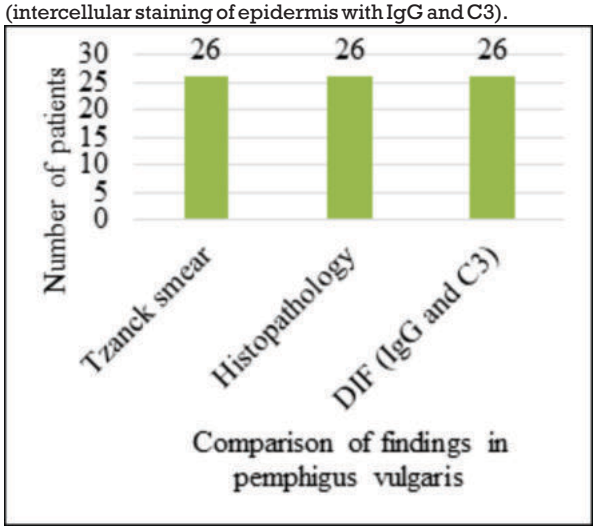

Figure 1: Comparison of Tzanck smear, histopathology and DIF in pemphigus vulgaris (n=27)

Out of 21 patients with bullous pemphigoid, 2 (9.52%), 20 (95.24%), and 19 (90.48%) had positive findings on Tzanck smear, histopathology, and DIF (Linear staining of basement membrane zone with IgG and C3), respectively.

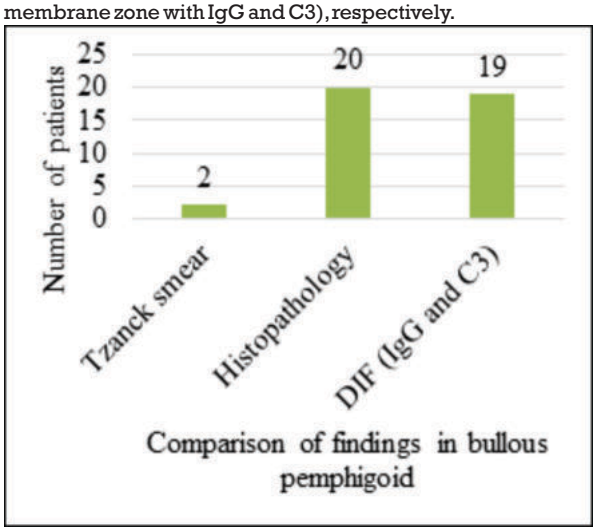

Figure 2: Comparison of Tzanck smear, histopathology and DIF in bullous pemphigoid (n=21)

All patients with pemphigus foliaceous (n=4), had positive findings on Tzanck smear, histopathology and DIF (intercellular staining of epidermis with IgG and C3, appears to be more intense in upper epidermis).

Table 2: Diagnostic Performance Of Tzanck Smear Compared To HPE And DIF.

Parameters	Tzanck smear vs HPE	Tzanck smear vs DIF
Sensitivity	61.54% (47.02% to 74.70%)	63.27% (48.29% to 76.58%)
Specificity	-	66.67% (9.43% to 99.16%)
Positive Predictive Value	100.00% (89.11% to 100.00%)	96.87% (83.78% to 99.92%)
Negative Predictive Value	0.00% (0.00% to 16.84%)	10.00% (1.23% to 31.70%)
Accuracy	-	63.46% (48.96% to 76.38%)

On evaluation, Tzanck smear findings of 32 patients were concordant with that of the HPE and hence were true positive cases. Moreover, 20 patients were confirmed on HPE and hence were false negative cases. There were no false positive and true negative patients. Thus, in the present study, when compared to HPE, Tzanck smear had sensitivity of 61.54%, positive predictive value (PPV) of 100%, and negative predictive value (NPV) of 0.00%.

Two patients were confirmed as negative on both Tzanck smear and DIF. One patient found positive on Tzanck smear was negative on DIF, hence was false positive. Finally, 18 patients found negative on Tzanck smear was positive on DIF (false negative).

Thus, in the present study, when compared to DIF, Tzanck smear had sensitivity of 63.27%, specificity of 66.67%, PPV of 96.87%, NPV of 10.00%, and diagnostic accuracy of 63.46%.

Table3: Diagnostic Performance Of Tzanck Smear Compared To HPE And DIF In Individual Immunobullous Disorder.

Disorder	Parameters	Tzanck smear vs HPE	Tzanck smear vs DIF
Pemphigus vulgaris (n=27)	Sensitivity	100.00%	100.00%
	Specificity	100.00%	100.00%
	PPV	100.00%	100.00%
	NPV	100.00%	100.00%
	Accuracy	100.00%	100.00%

Bullous pemphigoid (n=21)	Sensitivity	9.52%	10.53%
	Specificity	-	100.00%
	PPV	100.00%	100.00%
	NPV	0.00%	10.53%
	Accuracy	-	19.05%
Pemphigus foliaceous (n=4)	Sensitivity	100.00%	100.00%
	Specificity	-	-
	PPV	100.00%	100.00%
	NPV	-	-
	Accuracy	-	-

On evaluation of patients with Pemphigus Vulgaris, findings of 26 patients were concordant with that of the HPE, true positive cases. Moreover, 1 patient was negative on DIF.

When compared to HPE and DIF, Tzanck smear had sensitivity of 100.00%, specificity of 100%, PPV of 100%, NPV of 100.00%, and accuracy of 100% in diagnosis of pemphigus vulgaris.

There were no false positive and true negative patients among the patients with bullous pemphigoid. When compared to HPE, Tzanck smear had sensitivity of 9.52%, PPV of 100%, and NPV of 0.00% in diagnosis of bullous pemphigoid. Tzanck smear had sensitivity of 9.52%, PPV of 100%, and NPV of 0.00% in diagnosis of bullous pemphigoid when compared to DIF.

Among the patients with pemphigus foliaceous, there were no false positive, false negative, and true negative patients. When compared to HPE and DIF, Tzanck smear had sensitivity of 100%, and PPV of 100% in diagnosis of pemphigus foliaceous.

DISCUSSION:

Tzanck smear is based on the pathogenic process of acantholysis.[6] Due to the disintegration of the intercellular bridges, epidermal cells lose their ability to coordinate throughout this phase.[9] The Tzanck smear is frequently used to support the diagnosis of several conditions, including the pemphigus group of diseases and herpetic infections. To use this technique to its fullest potential, it is crucial to evaluate Tzanck smear findings in conjunction with sufficient clinical data.[10]

In the present study, the sensitivity of Tzanck smear was found to be 61.54% with 95% CI of 47.02% to 74.70% and PPV was 100% with 95% CI of 89.11% to 100.00% when compared to HPE. When compared with DIF, sensitivity, specificity and accuracy were observed to be 63.27%, 66.67% and 63.46% respectively. The PPV and NPV of Tzanck smear were 96.87% and 10% respectively when compared to DIF. In a study done by Chandrashekhar et al , Tzanck smear had a 77.4% sensitivity for detecting the intraepidermal immunobullous group of disorders. Since there was no false positivity recorded in the study population, the Tzanck smear specificity and PPV in diagnosing the same was found to be both 100%.[8] The Tzanck test had an 88.24% diagnostic accuracy in study by Basu et al. [11] In comparison to tumoral lesions, the diagnostic reliability of Tzanck smear was higher in erosive vesiculobullous and granulomatous lesions according to Eryılmaz et al. The kappa value for vesiculobullous lesions was found to be 0.79 with 95% CI 0.66-0.91.[12] In study by Aneesh et al, 81.57% (31/38) of all immunobullous diseases were shown to have acantholytic cells on the Tzanck smear.[1]

The varying results in different studies may be due to these studies were conducted in different geographical areas. The epidemiology of these diseases can be different in different populations. The diagnostic accuracy of Tzanck smear in some studies were compared with clinical diagnosis. Some studies compared Tzanck smear with HPE. [7,11]

In the present study, diagnostic value of Tzanck smear were also analysed in individual immunobullous disorders. The most common immunobullous disorder in the present study was Pemphigus vulgaris (n=27) followed by Bullous pemphigoid (n= 21). There were only four patients of Pemphigus foliaceous. The sensitivity, specificity, PPV, NPV and accuracy of Tzanck smear in diagnosing pemphigus vulgaris was 100% each respectively when compared to HPE as well as DIF. The sensitivity and PPV of Tzanck smear for pemphigus foliaceous was 100% when compared to HPE and DIF.

The 28 pemphigus cases in the study by Yaeen et al were all investigated with the Tzanck test, histological analysis, and direct immunofluorescence, with respective positive rates of 71.4%, 78.6%, and 71.4%. [6] Histological findings based on level of split were positive in all patients of pemphigus vulgaris whereas DIF was positive in 93.54% of patients. Tzanck smear was shown to be more efficacious, with 100% sensitivity.[1] Compared to DIF, Tzanck smear sensitivity was only 73% in study by Shaheen et al. [13] In 22 of the 29 patients of the pemphigus group of disorders, the Tzanck smears supported the clinical diagnosis.[10] Tzanck smear sensitivity and specificity for pemphigus were 100% and 43.4%, respectively, according to Durdu et al. [14] Most of the studies have shown the high sensitivity of Tzanck test for diagnosing pemphigus. From this we can conclude that Tzanck test can be used as early diagnosis of pemphigus.

The sensitivity of Tzanck smear in diagnosing bullous pemphigoid was 9.52% and 10.53% when compared to HPE and DIF respectively in present study. The specificity of Tzanck smear was found to be 100% when compared to DIF. Similar results were shown by Yaeen et al, the sensitivity and specificity of Tzanck smear were found to be 11.11% and 100% respectively.[6] Tzanck smears were negative for acantholytic cells in bullous pemphigoid in another study by Aneesh et al.[1] Similar findings were observed in the study Heera et al. and Kumar et al.[7, 10] Tzanck smear can be used to distinguish it from the pemphigus group of illnesses.[10]

The key advantage of Tzanck smear test is that it is cheap, easy, and quick procedure that does not need any specialised laboratory equipment. It is especially useful when performing a biopsy is challenging.[13] The drawbacks of the Tzanck smear are that if slides are incorrectly made from a crusted vesicle, representative material might not be obtained. This can also happen if the base of lesion is not scraped properly. Poorly preserved cells might sometimes resemble cancerous cells, giving the erroneous impression.[15]

CONCLUSION:

Tzanck smears are useful in providing a preliminary diagnosis of pemphigus and for distinguishing pemphigoid from pemphigus diseases, which can help patients receive treatment earlier. Regular use of the test along with a thorough clinical history can aid in improving the identification of various skin conditions.

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