**Original article:**

**Diagnostic utility of Tzanck Smear in various cutaneous skin infections**

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**Abstract:**

**Aim:** Tzanck smear is a simple test that gives reliable information in many of skin lesions and can be used for quick, presumptive diagnosis of a number of cutaneous infections. The aim of this study was to see the usefulness of tzanck smears in the diagnosis of cutaneous skin infections

**Materials and Methods:** Retrospective analysis of all tzanck smears received during the time period of (January 2016- March 2018), was carried out. Aspirates were collected from the clinically suspected skin lesions, smears were prepared and stained with Giemsa stain and evaluated,The smears were observed with the clinical correlation of the diagnosis of patient.

**Results:** A total of 100 tzanck smears were performed during our study period. Out of 19 cases of vesiculobullous lesions the tzanck smear positivity was seen in 4 cases , out of 35 Herpes viral infection cases 9 were positive by tzanck smear. Phemphigus group showed positivity of 15 out of 40 cases.Others (Pellagra, papillosis, Steven Johnson syndrome, Molluscum contagiosum, Contact dermatitis ) 6 cases were also included in the study which showed no positivity to tzanck smears. In our study the positivity of Tzanck smears was 28%.Most of the patients were on antiviral therapy Acyclovir and responded to treatment.

 **Conclusion:** Tzanck smear test is a rapid, inexpensive and useful test to aid in the diagnosis of clinically suspected vesico bullous, pustular and erosive skin lesions. It also serves as a useful adjunct to histopathological evaluation of skin biopsies, especially immuno-bullous skin disorders.

**Keywords:** Tzanck smear, pemphigus vulgaris, herpes infection

**Introduction-**

Cytodiagnosis is the study of the characteristics of an individual cell, and tzanck smears form an important aspect of this. The mechanism of acantholysis forms the basis of Tzanck smears, where there is breakdown of the intercellular bridges between the epidermal cells. This results in the intact cells which tend to become rounded, and are known as acantholytic cells.(1,2) Tzanck smears were first introduced in 1947 by Arnault Tzanck, for diagnosing vesico-bullous lesions of the skin, such as herpes simplex. It is now being widely used for other skin dermatoses as well such as vesiculo and immunobullous disorders and cutaneous infections.(3,4,5)

The advantages of the test are that it is fast, simple and inexpensive. It causes less patient discomfort and gives a rapid confirmation of herpetic lesions of the skin. Pemphigus group of diseases can be confirmed by the presence of acantholytic cells. The smear is taken from a lesion by gently scraping it and transferring the obtained material onto a clean microscope slide. It is then air dried and routinely stained with Romanowsky stain such as May-Grunwald-Giemsa stain. If PAP stain is used, the slide should be immediately fixed in alcohol.(3,6)Being the largest organ, the skin can be easily subjected to exfoliative cytology. However it is less routinely done as compared to other fields such as gynaecology, urology and endocrinology(3,6) .

Studies reporting the diagnostic value of Tzanck smear in various erosive and vesiculobullous leisions are limited, especially in Indian Literature.(2)Tzanck smear cannot substitute the standard diagnostic methods , but can help in establishing the clinical diagnosis with ease and rapidity and serve as an adjuvant tool to the diagnostic method(2), Thus being simple and cheap procedure , more studies will help in validitating the usefulness of Tzanck smear as a routine procedure in vesicullobullous disorders.(7)

**Aims and Objectives:**

1.The aim of this study was to highlight the use of tzanck smear as a diagnostic tool in the diagnosis of cutaneous skin lesions, in our tertiary care hospit

2.To confirm the diagnosis of herpes Skin Infections HSV 1and 2 by (HSV1+2 ) IgM Antibody by ELISA.

**Materials and methods:**

This is a hospital based retrospective study . All the Tzanck smears received for a period (Jan 2016 to March 2018) were included in the study. All Patients visiting the outpatient department, in the Department of Dermatology and suspected of having cutaneous infections such as herpes simplex, herpes zoster, varicella, pemphigus groups of disorders etc. were included in the study. The detailed clinical histories an diagnosis of all the patients were taken. The ages of the patients ranged from 1 year to 62 years.

Tzanck smears were prepared by gently scraping from the base of a recent lesion such as blister or vesicle. Samples were taken from a fresh vesicle, in cases of suspected viral infection. In case of the other lesions, the roof of the blister was opened, and the floor of the lesion was scraped with using a scalpel. The obtained material was smeared onto a single clean microscopic slide. The smear was then air dried and stained using Modified Giemsa stain.

Following staining, the slides were examined under light microscope for the identification of acantholytic cells, Multinucleated Giant cells and Ballooning of cells n Eiosinophils etc.

**Giemsa stain:**
It is a solution containing azure II-eosin, glycerin and methanol. The stain is composed of methylene blue eosinate, azure A eosinate, azure B eosinate, and methylene blue chloride.

**Method:** The commercially available Giemsa stain solution is diluted 1:10 with distilled water, and the diluted solution is poured over the smear and kept for 15 minutes. Then it is washed with water and examined under the microscope. The stained nuclei may vary in color from reddish blue to purple to pink. The cytoplasm stains bluish.(3)

 The stained smears were subsequently examined under light microscope for identification of multinucleated giant cells , acantholytic cells, inflammatory cells.

In our study suggestive diagnosis of pemphigus was given after observing many acantholytic cells and typical Tzanck cells or multinucleated giant cells. Numerous eosinophils were seen in addition to acantholytic cells in cases diagnosed as pemphigoid lesions .

**Pemphigus vulgaris**:  A typical Tzanck cell  or acantholytic cell is a large round keratinocyte with a hypertrophic nucleus, hazy or absent nucleoli, and abundant basophilic cytoplasm. The basophilic staining is deeper peripherally on the cell membrane ("mourning edged" cells) due to the cytoplasm's tendency to get condensed at the periphery, leading to a perinuclear halo.(3)

**Pemphigoid**: In these conditions, the findings of a Tzanck smear are non-specific and there are no acantholytic cells. The smear only serves to readily rule out pemphigus. Bullous pemphigoid shows scarcity of epithelial cells and an abundance of leukocytes, particularly eosinophils with leukocyte adherence. (3)

 **Herpes simplex, varicella, herpes zoster**:Infection by the herpes group of virus can be rapidly and reliably diagnosed by a Tzanck test. The typical features include characteristic multinucleated syncytial giant cells and acantholytic cells . The cells appear as if they have been inflated ("ballooning degeneration") and sometimes may grow tremendously, 60-80 m in diameter. The giant cells often have a tadpole, bipolar, or irregular teardrop shape with a smooth external contour, in sharp contrast to the jagged configuration of sheets of abraded normal squamous epithelium. Syncytial giant cells contain multiple nuclei (many with 8 or more) that exhibit nuclear molding, so that the nuclei fit together in a jigsaw puzzle like fashion. The nuclei show great variation in shape and size. Intranuclear inclusion bodies surrounded by clear halo are characteristic of herpetic infection, but are often difficult to find.(3)

 

**Picture-1 and 2 Acantholytic cells suggestive of Phemphigus vulgaris.**



**Picture 3 and 4 Multinucleated giant cells suggestive of Herpes simplex, varicella and herpes zoster infections.**



**Picture 5 Ballooning of cells in Herpes infections.**

ELISA test was performed on Herpes Samples according to manufacturer’s instructions. ELISA kit used was NovaLisa HSV1+2 IgM by NovaTech Immunodiagnostica. (8)

**Results:**

A total of 100 Tzanck smears were performed during the study period. Most of the patients presented with vesiculobullous lesions and pemphigus group of infections and Herpes viral infections. Out of 100 cases , major group was of pemphigus group (29 cases) out of which 11 cases (37.9%) were positive for tzanck smear which revealed plenty of acantholytic cells followed by Herpes viral Infections(35 cases ) out of which 9 cases (25.7%) showed Multinucleated giant cells. Bullous Pemphigoid 11 cases out of which 4 cases(36.3%) showed positivity to tzanck smear which revealed eoisinophils. Among the study group , Vesicullobullous lesions (19 cases) out of which 4 cases (21%) were positive for tzanck smear which revealed Multinucleated Giant cells.

Other group in our study (Pellagra, papillosis, Steven Johnson syndrome, Molluscum contagiosum, Contact dermatitis ) 6 cases were also included in the study which showed no positivity to tzanck smears.

Overall Tzanck smear positivity in 100 cases was 28%.

Most of the patients were on antiviral therapy Acyclovir and responded to treatment.

**Table1 : Details of Tzanck smear diagnosis**

|  |  |  |
| --- | --- | --- |
| Sr. No | Tzanck smear Diagnostic Category | No of Cases |
| 1. | Genital Herpes | 20 |
| 2 | Oral Herpes | 3 |
| 3 | Herpes zoster | 19 |
| 4 | Pemphigus vulgaris | 20 |
| 5 | Pemphigus foliacius  | 5 |
| 6 | Pemphigus vegetans | 4 |
| 7 | Bullous pemphigiod | 11 |
| 8 | Vesiculobullous lesions | 19 |
| 9 | Varicella | 3 |
| 10 | Others | 6 |
|  | Total | 100 |

**Table: 2 Tzanck smear findings in various group of disorders**

|  |  |  |
| --- | --- | --- |
| Sr. no | Viral Infections | Tzanck smear findings (%) |
| Positive  | Negative | Total no. of cases |
| 1. | Genital Herpes | 4(20%) | 16(80%) | 20 |
| 2 | Oral Herpes | 1(33.4%) | 2(66.7%) | 3 |
| 3 | Herpes zoster | 3(33.4%) | 6(66.7%) | 19 |
| 4 | Pemphigus vulgaris | 8(40%) | 12(60%) | 20 |
| 5 | Pemphigus foliacius  | 1(20%) | 4(80%) | 5 |
| 6 | Pemphigus vegetans | 2(50%) | 2(50%) | 4 |
| 7 | Bullous pemphigiod | 4(36.3%) | 7(63.6%) | 11 |
| 8 | Vesiculobullous lesions | 4(21%) | 15(78.9%) | 19 |
| 9 | Varicella | 1(33.4%) | 2(66.7%) | 3 |
| 10 | Others | 0(0%) | 6(100%) | 6 |
|  | Total | 28 | 72 | 100 |

A total of 35 herpes samples were screened for HSV1+2 IgM Antibodies by ELISA test. All the 35 samples were turned out to be negative for HSV1+2 IgM Antibodies by ELISA test. One case of Herpes simplex viral infection was positive by Tzanck smear by microscopy which revealed multinucleated giant cells in our laboratory was confirmned by positive IgM ELISA for HSV 1+2 Antibody from private laboratory. This sample was from a new born baby whose mother had herpes infection and new born baby had herpes leisons all over the body.

**Discussion:**

Tzanck smear has a widespread used in the diagnosis of especially the pemphigus groups of disorders as well as herpetic infections. It is important to interpret tzanck smear findings, along with adequate clinical findings, in order to use this procedure. Vesico-bullous lesions of skin are a heterogeneous group of disorders, where most cases are due to a primary disorder such a pemphigus. However it can also occur secondarily due to a number of unrelated conditions, which require histopathological examination for confirmation.(**9,10)** Tzanck smear is mostly helpful in providing presumptive diagnosis of pemphigus vulgaris when lesion is not available for biopsy or the disease is in early stage**( 11)** Pemphigus group of lesions show typical acantholytic cells. .(**3)** Other bullous lesions such as pemphigoid, show an absence of acantholytic cells with a predominance of inflammatory cells.(**4)** Similar findings were obtained in our study. The findings in bullous pemphigoid are non-specific and the tzanck smear can be used to differentiate the same with pemphigus group of disorders.(**7)** The use of direct immunofluoresence can be used to increase the specificity of this test.(**4)**

IgG deposits around the acantholytic cells in pemphigus was a finding reported in one study.(**1)** In another study the positivity rate regarding the diagnosis of pemphigus group of lesions was 71.4%, 78.6%, and 71.4% using Tzanck test.(**1)** Shaheen et al reported IgG immunoreactant in 10% of pemphigus patients, followed by C3 and IgM.(**12)**

In our study the positivity rate regarding the diagnosis of pemphigus group was 37.5% by Tzanck smear**.**  In our study the positivity of Tzanck smears was 28%.Viral infections are most of times diagnosed based on clinical grounds, tzanck smears are significantly heipful in its diagnosis.(**1)** Typical herpetic changes include multinucleation and crowding of the nuclei, nuclear moulding, peripheral margination of the nuclear chromatin, and ground glass appearance of the nuclei. Intranuclear inclusions with a prominent halo, ballooning degeneration and inflammatory cells can be seen.(**2,1,13)**

In our study, the findings of multinucleation and acnatholytic cells were seen in cases of clinically suspected herpetic infections. In case of Bullous Pemphigoid and Pemphigus vegetans Eiosinophils were seen abundantly with more predominance of inflammatory cells and absence of Acantholytic cells.

The sensitivity and specificity of Tzanck smears has been compared with other techniques like polymerase chain reaction (PCR).Ozcan et al. reported a Tzanck smear sensitivity and specificity of 76.9% and 100%, in herpetic infections when compared with PCR in their study.(**1,13)** The other uses of tzanck smears are used to heip in the diagnosis of cutaneous infections like molluscum contagiosum and leishmaniasis, granulomatous dermatitis, genodermatoses such as Hailey-Hailey disease, autoimmune disorders and tumours (basal and squamous cells carcinomas).(**1,6,14,15)** Also fungal infections such as Candida and Aspergillus can be identified based on the morphological appearance of their hyphae.(**1,15)**

The limitations of Tzanck smear are that if the clinical material is scanty and may not be available on the slides, If the slides are improperly prepared from a crusted vesicle. This can also result if the base of the lesion is not adequately scraped , then microscopic results can be affected . Poorly preserved cells can sometimes resemble neoplastic cells, leading to a wrong impression.(**1)**

The main advantage is that Tzanck smear is an inexpensive, simple, and rapid test and does not require any specialized laboratory equipment. It is especially helpful in cases where biopsy is difficult to perform.(**12)**

In our study, the findings of the Tzanck smear correlated with the clinical symptoms, thus proving it to be a valuable adjunct in a variety of dermatoses and cutaneous iftections especially vesibo-bullous and herpetic lesions.

 In our study all the 35 herpes samples which were subjected to Elisa were turned out to be negative. The reason behind the negative results would be the recurrent infections caused by Herpes simplex virus. (**1,16).** Some of the studies have stated that the low IgM positivity and High IgG positivity may be due to the short duration of IgM persistence following the infection or due to the treatment with antiviral agents earlier in the course of disease(**1,16).**

**Conclusion:**

Tzanck smears are helpful in giving a presumptive diagnosis of pemphigus, as well as differentiating pemphigoid from pemhigus disorders, which can help in early treatment for patients. Routine use of the test in addition to adequate clinical history can aid the diagnosis of diverse cutaneous skin lesions.

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