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Effectiveness of Tzanck Smear in various dermatosis – A Cytodiagnostic Approach

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Abstract:
Background : *Skin – the organ of human body with maximum surface area has accounted for number of lesions, broadly studied under the category of "Dermatosis". With the increasing incidence there is a need for improvement and detection of all these dermatosis in an early stage so that the treatment can more effective with minimum morbidity. At times histopathological features are not sufficient to determine the nature of dermatosis due to sampling bias and discomfort to patients during biopsy procedure. Early diagnosis becomes important to have clinico-cytological and cytohistological correlation. Histopathological findings of Tzanck Smear are very helpful in early diagnosis. Since the technique is economical and causes no discomfort to patients, we have selected this study to evaluate the effectiveness of Tzanck Smear in various dermatosis.*
Materials & Method: *The present study consists of 50 cases of various dermatosis from our institute. The details of clinical findings and investigations of Tzanck Smear and Biopsy were obtained in each case using the demographic data, by inspection and palpation of the lesion. Tzanck Smears were prepared, stained with Leishman's Stain, and observed under microscope (40X & 100X). Besides this Histopathological diagnosis was also done by Tissue Processing and staining by Haematoxylin & Eosin stain.*
Results : *Out of 50 cases, in 90% (45 cases) an accurate cytodiagnosis was made by Tzanck Smear. Histopathology was not available in 50% (25 cases) which includes non – availability of biopsy specimen from 40% (20 cases) of Herpes Infection. In 50% (25 cases) clinical, Tzanck Smear diagnosis and histological findings were confirmed.*
Conclusion: *Tzanck Smear proves to be a highly selective tool for early diagnosis in various dermatosis.*
Key words: *Immunobullous disorders, Genodermatosis, Leishman's Stain, Biopsy, Acantholysis*

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I. Introduction

Dermatosis is the group of lesions that includes immunobullous lesions, cutaneous infections, genodermatosis and cutaneous tumors. Its incidence is increasing despite the progress of diagnosis and treatment of all this dermatosis. [1, 2]

Methods to culture microbes (viruses) and doing biopsies take few days to a few weeks in reporting when there is a certain requirement of early help from the pathologist. [1]

It is important that these lesions are diagnosed early and accurately by a method which can have clinico-cytological correlation as well as cytohistopathological correlation. So that the need and expectations of the clinicians for early diagnosis from the pathologist can be satisfied. [3,4]

TZANCK SMEAR is the cyto-diagnostic method for the various dermatosis. It reveals especially ACANTOLYTIC CELLS (Tzanck cells).

Tzanck cell is a keratinocyte with a hypertrophic nucleus, hazy or absent nucleoli and the basophilic staining has a tendency to get condensed peripherally on the cell membrane, leading to a perinuclear halo.

Tzanck smear can also reveal INFLAMMATORY CELLS, MULTINUCLEATED GIANT CELLS, INCLUSION BODIES, CORPS RODS and GRAINS as well as ISOLATED SQUAMOUS CELLS. [1, 2, 3, 4, 5, 6, 7, 8, 9]

The technique is very cheap, easy to perform and does not cause any discomfort to the patient. It is particularly very important when surgical excision is not easy due to small size of the lesion or due to lack of consent from the patient for excision or inapproachable due to site of lesion. This technique also decreases the follow up drop out ratio due to OPD availability. Recent reports suggest that Tzanck smear has a very high COST EFFECTIVE RATIO, which is very important in a developing country like INDIA.

TZANCK SMEAR has a diagnostic value for **Pemphigus Vulgaris**, **Toxic Epidermal Necrolysis**, **Bullous Pemphigoid**, **Herpes Simplex**, **Varicella**, **Herpes Zoster**, **Molluscum Contagiosum**, **Leishmaniasis**,

Hailey-hailey disease, **Darrier disease**, **Vascular** and **Pustular Dermatosis** in neonates, **Basal cell epithelioma** and **Squamous cell carcinoma**. [1, 2]

II. Aims & Objectives

- To determine the applicability of TZANCK SMEAR for evaluation of acantholytic cells in various dermatosis.
- To obtain acantholytic cells as well as other useful cytological findings in various dermatoses from TZANCK SMEAR.
- To correlate different clinical & cytological findings from TZANCK SMEAR with histopathological findings

III. Literature Review

Cytology is the study of individual cells and their intrinsic characteristics and functions. Cytology was first used in cutaneous disorders by Tzanck in 1947, for the diagnosis of vesiculobullous disorders particularly herpes simplex. [1, 2, 9]

A. Cytodiagnosis Of Immunobullous Disorders

Pemphigus [1, 2, 4, 6, 8, 9, 10, 11, 12, 13, 14]

Tzanck test is very useful for the diagnosis of Pemphigus, particularly in the early stages of oral Pemphigus where a biopsy is uncomfortable to the patient and of little help in diagnosis. A typical Tzanck cell is a large round keratinocyte with a hypertrophic nucleus, hazy or absent nucleoli, and abundant basophilic cytoplasm.

Acantholytic cells that are no longer attached to other epithelial cells lose their polyhedral shape and characteristically become rounded.

Bullous Pemphigoid (BP), Stevens - Johnson Syndrome (SJS) and Erosive Lichen Planus [1, 2, 8, 9, 10, 11, 12, 13, 15]

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 leucocytes, particularly eosinophils with leukocyte adherence.

SJS and Lichen Planus may show altered or necrotic keratinocytes, leukocytes, fibrin filaments and rare fibroblasts.

B. Cytodiagnosis Of Cutaneous Infections

Herpes Simplex, Varicella, Herpes Zoster[1,2,4,5,7,8,9,10,11,12,13,14,15,16]

Infection by the Herpes group of virus can be rapidly and reliably diagnosed by a Tzanck test. It may, however, be impossible to distinguish between these conditions based on cytodiagnosis features.

The typical features include characteristic multinucleated syncytical giant cells and acantholytic cells. The cells appear as if they have been inflated (Ballooning Degeneration) and sometimes may grow tremendously in diameter.

Molluscum Contagiosum[1,2,4,8,9,10,11,12,13,14,15,16]

Tzanck smear reveals the presence of diagnostic intracytoplasmic Molluscum bodies (Henderson - Patterson bodies), the largest known inclusion bodies.

C. Cytodiagnosis Of Genodermatosis

Hailey – Hailey disease [1, 11, 12, 13]

Cytodiagnosis can easily differentiate Hailey – Hailey disease from Impetigo, Flexural Psoriasis or Eczema, which can closely mimic this genodermatosis. A Tzanck smear shows multiple acantholytic cells.

Darier disease [1, 4, 13]

Cytology in Darier disease reveals "corps ronds" and "grains". "Corps ronds" are isolated keratinocytes with a round shape and an acidophilic cytoplasm, which is retracted from the nucleus and denser peripherally (Mantle cells). The grains are seen as small, hyaline, acidophilic ovoid bodies resembling pomegranate seeds.

Vesicular and Pustular Dermatosis in neonates [1, 9]

Smears of pustules in transient neonatal pustulosis and infantile acropustulosis show predominance of neutrophils. Smears of pustules in eosinophilic pustulosis show plentiful eosinophils.

D. Cytodiagnosis Of Cutaneous Tumors

Basal Cell Epithelioma [1, 10, 11]

A Tzanck smear offers a high degree of reliability in the diagnosis of basal cell epithelioma.

Squamous Cell Carcinoma [1, 10, 11, 12, 13]

Cytology is helpful in the nodular, soft or ulcerated non-keratotic varieties of squamous cell carcinoma, but not useful in keratotic or verrucous lesions. Tzanck smear prepared from the ulcerated area show irregular clump of relatively cohesive non keratinizing cells with variation in chromatin density. Nuclear alterations like hypertrophic, hyperchromatic, lobated or multiple nuclei, and abnormal mitoses are seen on higher magnification.

Paget's disease [1, 2, 4, 9, 11, 12, 13]

Paget's cells can be easily visualized on Tzanck smear. They occur singly or in small groups, and are round to oval cells with amphophilic, vacuolated cytoplasm and a hypertrophic nucleolated nucleus. They appear larger than keratinocytes. Special stains for epithelial mucin (mucicarmine periodic acid – sciff stain) can further corroborate the diagnosis by staining most Paget's cells.

Erythroplasia of Queyrat [1, 9, 13]

A smear shows polyhedral, spindle – shaped and round cells with "poikilokaryosis" (nuclear polymorphism relating to size, shape and staining), which is practically diagnostic for this intraepithelial carcinoma.

IV. Materials And Method [1, 2, 3, 9, 17, 18]

Preparation of Tzanck Smear:

- Tzanck smear is a very simple and rapid technique.
- For viral infections, samples should be taken from a fresh vesicle, rather than a crusted one, to ensure the yield of a number of virus infected cells.
- The vesicle should be unroofed or the crust removed, and the base scraped with a scalpel or edge of a spatula.
- The material is transferred to a glass slide by touching the spatula to the glass slide repeatedly but gently.
- The slide should be clean, since cells will not adhere to a slide marred by fingerprints.
- In the case of blistering disorders, the intact roof of the blister is opened along one side, folded back and the floor gently scraped.
- The material thus obtained is smeared onto a microscopic slide, allowed to air dry, and stained with Leishman's stain.
- Smearing Bulla fluid and inclusion of blood may lead to inappropriate results.
- For the cytodiagnosis of suspected tumors, any crust is removed from ulcerated tumors, and non – ulcerated tumors incised with a sharp, pointed scalpel (the incision should be superficial enough to avoid undue bleeding).
- A sample of tumor is then obtained with either a blunt scalpel or a small curette, and the tissue obtained is pressed between two slides.

Preparation of solution of Leishman's Stain:

- Air dry the film & flood the slide with the stain.
- After 2 mins, double the volume with water & stain the film for 5 – 7 mins.
- Then wash it in a stream of buffered water until it has acquired a pinkish tinge (up to 2 mins).
- After the back of the slide has been wiped clean, set it upright to dry.
- Mount it with DPX and cover it with glass cover slip.

Staining of Tzanck Smear for the cytodiagnosis of suspected tumor:

- Fix the smears with fixative
- Place in haematoxylin for 3 mins.

- Wash with water
- Decolorize with 1% Acid alcohol
- Wash with water immediately
- Place in running water for 10 mins.
- Place in 1% Eosin for 1 min.
- Wash with water
- Allow it to air dry & mount with D.P.X.

V. Observation

Present study consists of 50 cases of various dermatosis in which TZANCK SMEAR is evaluated for its cytodiagnostic value.

TABLE NO. : 1
AGE DISTRIBUTION IN VARIOUS DERMATOSIS

VARIOUS LESION V/S AGE GROUP IN YEARS	UPTO 14 YEARS	UPTO 40 YEARS	41 TO 60 YEARS	ABOVE 60 YEARS	MEAN AGE IN VARIOUS LESION
PEMPHIGUS	01	08	02	00	35.91
BULLOUS PEMPHIGOID	00	06	01	01	40.73
HERPES SIMPLEX	00	07	00	00	25.68
HERPES ZOSTER	01	08	03	00	28.68
MOLLUSCUM CONTAGIOSUM	05	02	01	00	17.81
SQUAMOUS CELL CARCINOMA	00	00	04	00	50.20

TABLE NO. 1 shows that Molluscum Contagiosum is more common in younger age group up to 14 years of life. Pemphigus lesion, Bullous Pemphigoid and Herpes infection are common in 15 to 40 years age group. While squamous Cell Carcinoma is more common in 41 to 61 years age group in our study.

TABLE NO. : 2
SEX DISTRIBUTION IN 100 CASES OF VARIOUS DERMATOSIS

SERIAL NO.	SEX	TOTAL NO. OF CASES	PERCENTAGES (%)
1.	MALE	27	54
2.	FEMALE	23	46

TABLE NO. : 2 shows that Males consist of 54% of the total 50 cases studied while 46% were Females. There is no significant difference in the Male: Female ratio in the cases of various dermatosis.

TABLE NO. : 3
SEX DISTRIBUTION IN VARIOUS DERMATOSIS

VARIOUS LESION V/S SEX GROUP	MALE	FEMALE	M:F RATIO
PEMPHIGUS	06	05	1.2 : 1
BULLOUS PEMPHIGOID	03	05	1 : 1.6
HERPES SIMPLEX	05	02	1 : 0.4
HERPES ZOSTER	05	07	1 : 1.4
MOLLUSCUM CONTAGIOSUM	04	04	1 : 1
SQUAMOUS CELL CARCINOMA	04	00	4 : 0

TABLE NO. : 3 shows that Herpes Zoster and Bullous Pemphigoid is more common in the Females while Squamous Cell Carcinoma is more common in Males.

TABLE NO. : 4
SITE DISTRIBUTION IN 50 CASES OF VARIOUS DERMATOSIS

SERIAL NO.	SITE	NO. OF CASES	PERCENTAGE (%)
1.	FACE	17	34
2.	NECK	02	04
3.	CHEST WALL & BACK	03	06

4.	ABDOMEN	00	00
5.	GENITAL	11	22
6.	LIMBS	09	18
7.	ALL OVER THE BODY	08	16

TABLE NO. : 4 shows that out of 50 cases 34% of the cases occurs on face which is the most common site while the second most common site (22%) is the genital region while significant percentages (16%) of the lesions occur all over the body.

TABLE NO. : 5

CYTOLOGICAL FINDINGS OF TZANCK SMEARS IN 50 CASES OF VARIOUS DERMATOSIS

SERIAL NO.	CLINICAL DIAGNOSIS	TZANCK SMEAR POSITIE FINDINGS	TZANCK SMEAR POSITIVE CASES
1.	PEMPHIGUS	Acantholytic cells, Hazy nucleoli	10
2.	BULLOUS PEMPHIGOID	No acantholytic cells, plenty of leucocytes	07
3.	HERPES SIMPLEX	Ballooning multinucleated giant cells	06
4.	HERPES ZOSTER	Ballooning multinucleated giant cells	11
5.	MOLLUSCUM CONTAGIOSUM	Henderson – Patterson Bodies	07
6.	SQUAMOUS CELL CARCINOMA	Atypical Squamous Cells	03

TABLE NO. : 6

CYTODIAGNOSTIC VALUE OF TZANCK SMEAR IN VARIOUS CLINICALLY SUSPICIOUS DERMATOSIS

SERIAL NO.	CLINICAL DIAGNOSIS	NO. OF CASES	TZANCK SMEAR POSITIVE CASES	PERCENTAGE OF TZANCK SMEAR POSITIVE CASES (%)	INADEQUATE MATERIAL
1.	PEMPHIGUS	11	10	90.90	01
2.	BULLOUS PEMPHIGOID	08	07	87.50	01
3.	HERPES SIMPLEX	07	06	85.00	01
4.	HERPES ZOSTER	12	11	91.00	01
5.	MOLLUSCUM CONTAGIOSUM	08	07	87.50	01
6.	SQUAMOUS CELL CARCINOMA	04	03	75.00	01
TOTAL		50	44	88.00	6

TABLE NO. : 6 shows that Tzanck Smear is a highly sensitive (88.00%) technique for cytodiagnosis of various dermatosis.

TABLE NO. : 7

CORRELATION OF CLINICAL DIAGNOSIS, POSITIVE TZANCK SMEAR FINDING & POSITIVE BIOPSY RESULT

SR. NO.	CLINICAL DIAGNOSIS	NO. OF CASES	TZANCK SMEAR POSITIVE	H.P. POSITIVE	% OF H.P. POSITIVE CASES	INADEQUATE MATERIAL
1.	PEMPHIGUS	11	10	10	90.90	01
2.	BULLOUS PEMPHIGOID	08	07	07	87.50	01
3.	HERPES SIMPLEX	07	06	NOT DONE	NOT DONE	07
4.	HERPES ZOSTER	12	11	NOT DONE	NOT DONE	12
5.	MOLLUSCUM CONTAGIOUS	08	07	07	87.50	04
6.	SQUAMOUS CELL CARCINOMA	04	03	03	75.00	01
	TOTAL	50	44	26	52.00	50

TABLE NO. : 7 shows Tzanck Smear results are highly comparable in context of positivity with clinical diagnosis & histopathology in Pemphigus, Bullous Pemphigoid, Molluscum Contagiosum and Squamous Cell

Carcinoma. Histopathology of Herpes infected lesion (Herpes Simplex and Herpes Zoster) were not available for the comparison due to non – compliance of the patients. The diagnosis was therefore based on clinical presentation and Tzanck Smear Morphology, which confirms the presence of Herpes Infection.

VI. Discussion (1, 2, 3, 9, 11, 19, 20, 21, 22)

In the present study of 50 cases of various dermatosis, different data like Age incidence, Sex incidence, Clinical diagnosis, Tzanck smear finding, Biopsy finding taken from the same lesion and their relationship with each other were obtained.

The result of the present study allows a reliable evaluation of the accuracy of Tzanck Smear in the early diagnosis of the various dermatosis.

VII. Summary And Conclusion

Tzanck Smear can aid in establishing the early clinical diagnosis and serve as an adjuvant to routine histological study.

Tzanck Smear is cheap, easy to perform and does not cause any discomfort to the patient.

Tzanck Smear is a quite rapid O.P.D procedure as compared to routine histopathology procedure. Thus early clinico – pathological correlation can be established which is very good for the treatment prospective.

Tzanck Smear is the cost – effective technique for the clinico – pathological correlation for the HERPES Infection where consent for the biopsy is difficult to be taken because of the patient's discomfort.

Although Tzanck Smear is not a substitute for standard histopathology, in the hands of experienced pathologist, Tzanck Smear is a sensitive & specific early diagnosis tool.

Despite the exponential growth & interest in dermatopathology and skin is the largest desquamating organ in the body, interest in the Tzanck Smear has been limited. Attention must be paid over Cytodiagnostic value of Tzanck Smear in various dermatosis, where ever possible.

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