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 acantholytic 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,
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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

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

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

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 syncytial 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

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

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

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.
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Vesicular and Pustular Dermatosis in neonates

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

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

Squamous Cell Carcinoma

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

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 – schiff stain) can further corroborate the diagnosis by staining most Paget’s cells.

Erythroplasia of Queyrat

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

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.
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- Wash with water
- Decolorize with 1% Acid alcohol
- Wash with water immediately
- Place in running water for 10 mins.
- Place in 5% 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 VS 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 VS 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 |

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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

CYTLOGICAL FINDINGS OF TZANCK SMEARS IN 50 CASES OF VARIOUS DERMATOSIS

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

TABLE NO. : 6

CYTODIAGNOSTIC VALUE OF TZANCK SMEAR IN VARIOUS CLINICALLY SUSPICIOUS DERMATOSIS

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

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

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

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

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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