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Accuracy and reliability of Tzanck smear compared to histopathology in ulcerative and nodular lesions of Skin

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Abstract

Background: Though cytopathology was an excellent diagnostic tool in routine dermatologic practice, studies relating to histopathological and cytological correlation in relation to ulcerative and nodular lesions are few. The objective of our study was to evaluate cytology as a quick non-invasive method for early diagnosis and to know the accuracy and reliability of Tzanck smear in various ulcerative and nodular skin lesions.

Materials and Methods: A total of 55 cases were chosen from dermatology out-patient department, who presented with ulcerative and nodular lesions. Tzanck smear examination and histopathological examination (incisional/ excisional/ punch biopsy) were done in each case. Keeping histopathological diagnosis as the gold standard test, sensitivity and positive predictive values were calculated to determine the accuracy of results.

Results: Out of 55 cases, 49 showed accurate diagnosis in cytological Tzanck smear examination. Majority of ulcerative and nodular lesions presented between 31-40 years and 11- 20 year age group. The sensitivity of cytology in diagnosing inflammatory lesions, benign neoplastic lesions and malignant neoplastic lesions was 91.6%, 83.3% and 88% respectively. Out of 25 cases clinically diagnosed as malignancy, squamous cell carcinoma was diagnosed in 8 cases, Basal cell carcinoma in 6 cases, Malignant melanoma in 7 cases, and one case each is diagnosed as Sebaceous carcinoma, histiocytosis, Mastocytosis and Erythroplasia of Queyrat respectively.

Conclusion: Tzanck smear is a rapid, non-traumatic, cost effective, safe and important diagnostic tool for the early diagnosis of cutaneous ulcerative and nodular lesions. Cytology can be used to differentiate inflammatory lesions from neoplastic, neoplastic as benign or malignant and definitively diagnose several specific malignant cutaneous lesions and aid in further course of treatment modalities.

Keywords: Tzanck smear, Sensitivity, Ulcerative, Nodular lesions

Introduction

The cytological test, otherwise the Tzanck smear test, was introduced by French author Arnault Tzanck, in his name in 1947.[(1)] Tzanck smear and skin biopsy form the fundamental basis for differential diagnosis in various clinically diagnosed dermatological lesions, thereby providing information to the dermatopathologist.[(1,2)] Tzanck smear was considered as an excellent diagnostic tool in routine dermatological investigations, and studies with histopathological and cytological correlation in relation to ulcerative and nodular lesions are few.[(2)] The objective of our study was to evaluate cytology as a quick non-invasive method for early diagnosis, and to the know the accuracy and reliability of Tzanck smear in diagnosis of various ulcerative and nodular skin lesions.

Materials and Methods

Study design: The present Prospective study was undertaken in the Department of Pathology in our institution, a tertiary health care centre, which was conducted over the period of 6 months from November 2015 to April 2016, after obtaining approval from the Institutional Ethical Committee.

Study Population: A total of 55 cases were chosen from dermatology out-patient department who presented with ulcerative and nodular lesions. Patients with vesiculo-bullous lesions were excluded from the study.

Collection of data: After detailed clinical history from the patients was taken, complete dermatological examination findings were recorded. Tzanck smear examination and histomorphological examination (incisional/ excisional/punch biopsy) were done in each case.

Tzanck smear preparation and interpretation: To get sample for crusted ulcerative lesions, crusts were removed after soaking the affected area either with normal saline or in distilled water for 10 minutes. 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. The smear was finally examined under light microscope for cytological findings.

Histopathological examination: Following post-operative excisional, incisional or punch biopsies, the samples were fixed in 10% buffered formaldehyde,

paraffin embedded and thin sections of around 4 microns were made and stained with hematoxylin and eosin method for analysis of histomorphological patterns and to arrive at a diagnosis based on the patterns. Ancillary investigations like special stains and immunohistochemistry markers were used if necessary. Cytological evaluation was done and results were correlated with histopathological and clinical findings.

Statistical analysis: The results obtained were analysed for statistical significance by using Fisher exact test. Keeping histopathological diagnosis as the gold standard test, sensitivity, specificity, positive predictive values and negative predictive values were calculated to determine the accuracy of results.

Results

A total of 55 cases were taken for both cytological and histopathological examination. Keeping histopathological diagnosis as the gold standard confirmatory test, cytological correlations were made. Out of 55 cases, 49 showed accurate diagnosis in cytological Tzanck smear examination. Majority of ulcerative and nodular lesions presented between 31-40 year and 11- 20 year age group (Table 1). Youngest patient was seven years old female child and oldest patient was 68 years old male in the study group. Comparatively, males slightly outnumbered females in this study.

Non-neoplastic lesions: In a total of 24 Non-neoplastic ulcerative and nodular lesions, there were seventeen cases of herpes infection (Herpes simplex and Herpes zoster), six cases of molluscum contagiosum and one case of scar endometriosis. Tzanck smear confirmed 15 cases as Herpes (both herpes simplex and zoster) which revealed balloon cells (ballooning degeneration), multinucleated giant cells and ground glass nuclei (bland inclusion containing nuclei), with nuclear moulding (Fig. 1 and 2). Histopathology differentiated Herpes Simplex (HS) from herpes Zoster (HZ). Average age was 12.5 years for HS and 20 years for HZ. Clinically, patients presented with painful ulcerative lesions with a haemorrhagic base. Six patients of molluscum contagiosum (MC) were observed, who presented with discrete papules with crusted centers. Cytology and histopathological examination showed presence of squamous cells with basophilic or eosinophilic round, cytoplasmic inclusion bodies with peripherally pushed nuclei. One case of endometriosis was noted in a 38 years old–female who presented with a tiny nodular lesion in the abdominal wall. Cytology and histopathology showed endometrial glands and stroma. Of all 24 cases, 22(91.6%) cases showed cytology positivity for all non-neoplastic lesions (Table 2).

Table 1: Age distribution of Ulcerative and nodular lesions

Age group in years	No of patients
0 - 10	08
11- 20	13
21- 30	06
31- 40	16
41- 50	07
51- 60	02
>60	03
Total	55

Table 2: Cytohistological correlation of ulcerative and nodular lesions of skin

Classification	Histopathological diagnosis	No of cases	Cytology correlation	Discordant	Sensitivity %
Non neoplastic	Herpes simplex and zoster	17	15	2	91.6%
	Molluscum contagiosum	6	6	-	
	Scar endometriosis	1	1	-	
	Total	24	22	2	
Benign Neoplastic	Epidermal inclusion cyst	4	3	1	83.3%
	Benign adnexal tumours	2	2	-	
	Total	6	5	1	
Malignant neoplastic	Squamous cell carcinoma	8	7	1	88%
	Basal cell carcinoma	6	5	1	
	Malignant melanoma	7	4	3	
	Sebaceous carcinoma	1	1	-	
	Mastocytosis	1	1	-	
	Histiocytosis	1	1	-	
	Erythroplasia of Queyrat	1	1	-	

	Total	25	22	3	

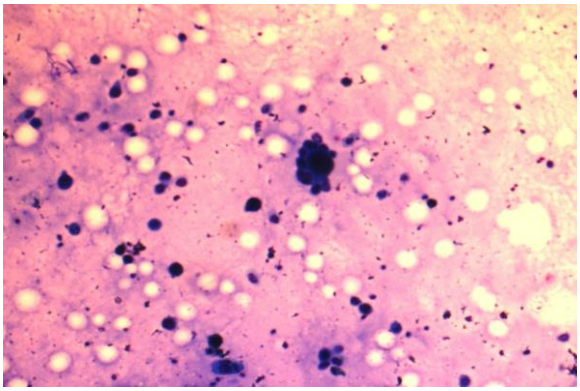

Fig. 1: Multinucleated syncytial giant cells along with few scattered acantholytic cells seen in Herpes (MGG, X10)

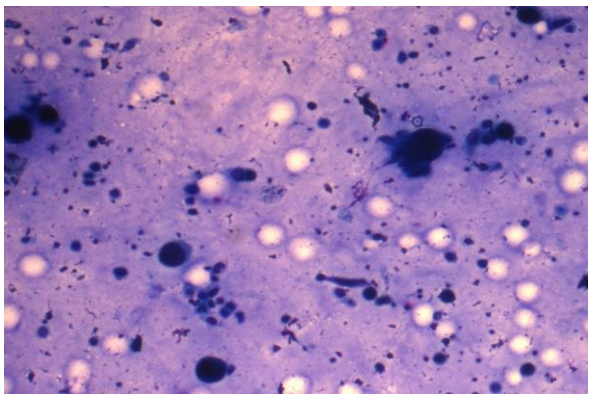

Fig. 2: Giant cells and acantholytic cells seen in a neutrophilic background in Herpes infection (MGG, X10)

Benign neoplastic lesions: In the present study, benign adnexal tumors and epidermal inclusion cysts were included under ulceronodular lesions (Table 2). Mean age of presentation was 23 years. Histopathological examination confirmed the two benign adnexal tumors as Eccrine poroma and nodular hidradenoma respectively. Out of all 6 benign neoplastic lesions, 5 (83%) showed Tzanck smear positivity. One case of clinically diagnosed epidermal inclusion cyst was inadequate for Tzanck smear diagnosis. On cytology, smears showed eosinophilic keratinous material with anucleate and nucleated squamous cells with cellular debris in the background (Fig. 3). Histology showed cysts filled with keratinous material and its wall lined by stratified squamous epithelium with an intact granular layer.

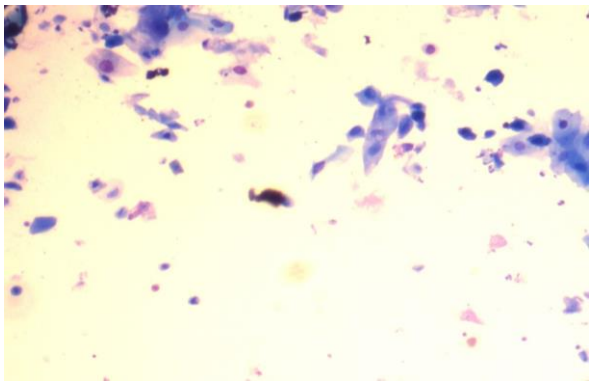

Fig. 3: Epidermal cyst: Nucleate and anucleate squamous cells in the background of keratinous debris (MGG, X10)

Malignant Lesions: Out of 25 cases clinically diagnosed as malignancy, squamous cell carcinoma was diagnosed in eight cases, Basal cell carcinoma in six cases, Malignant melanoma in seven cases and one case each diagnosed as Sebaceous carcinoma, Histiocytosis, Mastocytosis and Erythroplasia of Queyrat. Cytology showed smear positivity for 22 cases out of 25 cases with the sensitivity of 88% in all malignant skin lesions (Table 2).

Clinically, the lesions of squamous cell carcinoma were ulcerated, fungating and nodular growths with crusting and indurated margins. Out of 8 cases, 7 cases were confirmed by Tzanck smear which revealed malignant pleomorphic squamous cells with vesicular nucleus and prominent nucleoli (Fig. 4). Mean age of presentation was 38 years.

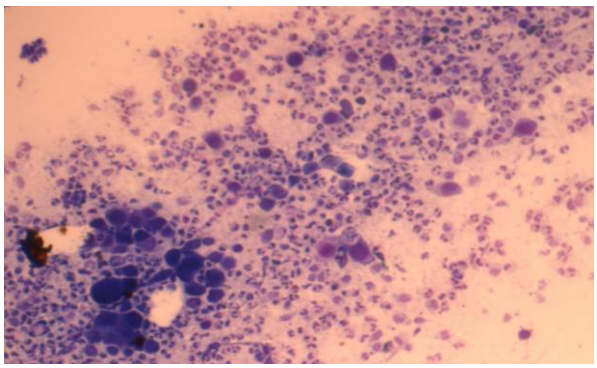

Fig. 4: Squamous cell carcinoma: Pleomorphic squamous epithelial cells with hyperchromatic nuclei with prominent nucleolus and abundant keratinization in clusters and singles. (MGG, X10)

Clinically, all six cases of Basal cell carcinoma (BCC) presented with multiple nodules with or without ulceration situated on one side of cheek, nose and over forehead. Tzanck smears revealed tumor cells in cohesive sheets having small, round to oval hyperchromatic nuclei with evenly distributed chromatin

and scanty cytoplasm. Histology revealed closely packed, small round to oval basophilic cells with scant cytoplasm, hyperchromatic nuclei with peripheral palisading. Cytology confirmed 5 cases with the sensitivity of 83.3%.

Seven cases of malignant melanoma clinically presented as hyperpigmented multiple mottled ulcerative and nodular lesions with irregular borders. Four cases, on cytological examination, showed discohesive pleomorphic tumor cells with eccentrically placed irregular hyperchromatic nuclei with abundant melanin pigment. In all seven cases, histology revealed bizarre plump melanocytes with prominent eosinophilic nucleoli and scattered pigment and melanophages.

A single rare case of sebaceous carcinoma was seen in a 45-year old female who presented with a nodular mass on the trunk, which was having both solid and cystic areas. Cytology showed cohesive clusters of epithelial cells with enlarged nucleus, prominent nucleolus, and abundant eosinophilic cytoplasm with decapitation secretions. Histology revealed sheets and trabecular pattern of malignant cells, having pleomorphic vesicular nucleus with prominent nucleoli and, eosinophilic, clear, vacuolated cytoplasm and abnormal mitotic figures.

Discussion

Non-neoplastic lesions: Clinical identification of herpes simplex is generally so straightforward that laboratory tests are not usually required. But Tzanck smear is a reliable tool in identifying Herpes simplex in ruling out other mimickers like herpetic gingivostomatitis, Kaposi varicelliform eruption.[3] The mean age incidence of herpes simplex in our study was 12.5 years which can be comparable to the study of Crumpacker et al.[4] Cytological features observed were in accordance with the findings of Graham *et al* and Sehgal et al.[5,6] In all six cases of Molluscum contagiosum, cytohistopathological correlation was 100 % concordant showing high sensitivity similar to the study of Patil et al.[7] A rare endometriosis case was seen which presented in the anterior abdominal wall as a nodular lesion and showed cytological features similar to the study of Srivatsava et al.[8]

Benign Neoplastic lesions: In all the six benign lesions studied, accurate diagnosis was made in five case and one case was found to be inadequate biopsy specimen for diagnosis. The overall positivity rate of Tzanck smear (83.3%) can be comparable with the study of Layfield et al.[9] Of the all benign neoplastic lesions, Epidermal cyst constituted 66.6%, the study of which can be compared to the study done by Srivastava et al in which epidermal cyst constituted 45.4 %.[8]

Malignant neoplastic lesions: Squamous cell carcinoma (SCC) was the most common malignant neoplastic lesion seen in our study comprising eight cases of a total of 25 malignant lesions studied. The lesions were seen distributed in the oral, genital and trunk regions, which were found in contrast to the studies reported by Dracopoulou et al. and Allen.[10,11] Cytological correlation of the eight cases confirmed as SCC in seven and one case proved to show moderate dysplasia on histopathological examination (87.5% sensitivity). The accuracy of Tzanck smear in the diagnosis of SCC was found less in the variants such as keratotic or verrucous lesions.[12] But in our study, the sensitivity of Tzanck smear was increased by pretreating the lesions of clinically suspected SCC cases with bland keratolytic compresses (sodium bicarbonate 3% soaked) for 30 minutes. This could dissolve the keratinic debris and could yield atypical cells in the deeper lesions.[13]

In all the six cases of BCC, Lesions were seen on the face, similar to the findings by Allen and Malberger et al.[11,14] BCC is the main indication for the Tzanck smear test because of both the frequency of the neoplasm and the high degree of diagnostic reliability offered by cytological examination.[15] Also this test is particularly useful in cases with multiple, superficial lesions where various methods of treatment such as cryotherapy, electrodessication, laser ablation, radiotherapy, photodynamic therapy, immunotherapy and chemotherapy are considered even before the possibility of histological confirmation.[15] Similar to the studies of Vivek kumar Dey et al, Layfield and Glasgow and Malberger et al,[9,14,16] high sensitivity (83%) with the correlation of cytological findings for BCC was observed in the present study.

In the present study, the mean age incidence of malignant melanoma was 32.5 years. It correlated with the study by Perry et al[17] who reported a major number of cases in the younger age group. However, this was in contradiction with the study of Hadju and Savino[18], which reported higher incidence of the tumor in elderly cases. The sensitivity rate (57%) for Malignant melanoma in the present study was low. This could be due to the lack of appreciation of presence of melanin pigment intracellularly in a large number of cells. Similar observations were made by Perry et al in his study.[17]

A rare case of Sebaceous carcinoma (SGC) over the eyelid was diagnosed in a 48-year-old female. The cytological findings observed in the smear were similar to those described by Gao et al.[19] The cytological study also confirmed each single cases of other rare lesions such as Mastocytosis, Histiocytosis and Erythroplasia of Queyrat. The cytological features observed in each case were similar to those studied by Gupta et al.[20]

Limitations of the study

Though Tzanck smear is helpful in the rapid diagnosis of malignant ulcerative and nodular lesions, it does not categorise tumor patterns or its subtype variants. The histologic characteristics influence clinical behavior, recurrence, and metastatic potential and therapeutic planning.[21] It can very well differentiate

benign from malignant lesions and therefore can aid in further course of management.

The sensitivity of Tzank smear in diagnosing inflammatory lesions, benign neoplastic lesions and malignant neoplastic lesions was 91.6%, 83.3% and 88% respectively. In a total of 55 cases (inflammatory and neoplastic lesions), accurate diagnosis was made by cytology in 49 cases (89%). Thus a considerably high degree of correlation was achieved between cytological and histological modalities of diagnosis, making Tzanck smear as one of the useful and rapid, diagnostic tools in diagnosing cutaneous ulcerative and nodular lesions.

Conclusion

To conclude, Tzanck smear is an important simple, rapid, non-traumatic, cost effective and safe diagnostic tool for early diagnosis of cutaneous ulcerative and nodular lesions. Tzanck smear can be used to differentiate inflammatory lesions from neoplastic lesions and neoplastic lesions as benign or malignant, and to diagnose definitively several specific malignant cutaneous lesions and to aid in further managing course of treatment modalities.

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