



Original Research Article

## A Clinicocytological Appraisal of Vesiculobullous and Noduloulcerative Lesions of Skin

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### ABSTRACT

**Introduction:** Cytopathology has proven to be of great help in the diagnosis of various skin lesions, however, studies relating to histopathological and cytological correlation are few. The objective of this study was to evaluate cytology as an early diagnostic tool in comparison to histopathology in various vesiculobullous and noduloulcerative lesions of skin.

**Material and methods:** The study material constituted 50 cases of vesiculobullous and noduloulcerative lesions of skin. A detailed history of the patients was taken and complete physical and dermatological examination findings including clinical diagnosis, were recorded. Cytological examination (Tzanck/FNAC/Scrapings) and histopathological examination (incisional/ excisional/punch biopsy) were done in each case.

Clinicocytological evaluation was done and results were correlated with histopathological findings. However, in case of vesiculobullous lesions, immunofluorescence findings were also correlated for further confirmation of the diagnosis

**Result:** Overall, the sensitivity of cytology in diagnosing vesiculobullous and noduloulcerative lesions of skin was 89.36%

**Conclusion:** Cytology has been proven as a reliable and informative tool in the diagnosis of various vesiculobullous and noduloulcerative lesions of skin. In comparison to biopsy techniques; it is a non traumatic, inexpensive, safe, well tolerated and a rapid diagnostic tool.

**Keywords:** Cytopathology, skin, vesiculobullous, noduloulcerative.

### INTRODUCTION

Cytology and skin biopsy form the basis of differential diagnosis in clinically similar dermatoses, thereby yielding important information to the pathologist and dermatologist. <sup>[1]</sup>

George Papanicolaou is considered the father of exfoliative cytology, but cytology was first used in cutaneous

disorders by Tzanck in 1947, for the diagnosis of vesiculobullous disorders, particularly herpes simplex. Since then cytology has been widely used by dermatologists for diagnosing various cutaneous dermatoses. <sup>[2]</sup>

Although not commonly employed, cutaneous cytology has become a useful tool for clinicians due to the development of

simplified procedures and staining techniques.<sup>3</sup>In many cases it is sufficient for definitive diagnosis and preferable to biopsy from areas such as face to avoid or minimise scarring, particularly in conjunction with topical treatment modalities in some types of skin cancer. Furthermore, cytological material is well suited for certain ancillary tests. It is a simple, inexpensive diagnostic method and is especially helpful when resources are limited.<sup>[3]</sup> Its various methods are aspiration cytology, imprint smear, exudate smear, skin scraping smear, and Tzanck smear. Cytopathology has proven to be of great help in the diagnosis of various skin lesions, however, studies relating to histopathological and cytological correlation are few. The objective of this study was to evaluate cytology as an early diagnostic tool in comparison to histopathology in various vesiculobullous and noduloulcerative lesions of skin.

## **MATERIALS AND METHODS**

The present study was carried out in the Department of Pathology in collaboration with Department of Dermatology, Pt. Bhagwat Dayal Sharma, Post Graduate Institute of Medical Sciences, Rohtak. The study material constituted 50 cases of vesiculobullous and noduloulcerative lesions of skin

A detailed history of the patients was taken and complete physical and dermatological examination findings including clinical diagnosis, were recorded. Cytological examination (Tzanck/FNAC/Scrapings) and histopathological examination (incisional/ excisional/punch biopsy) were done in each case. Cytological smears were prepared depending on the type of lesion. Tzanck smears were prepared for vesiculobullous lesions.

FNAC was done in cases of nodular lesions. Skin scrapings were obtained for superficial lesions such as those with superficial ulcers and ulcerated tumors.

One of the cytological smears was fixed in 95% ethanol and stained with Papanicolaou stain (Pap), others were stained with leishman stain.

Excisional, incisional and punch biopsies were obtained for histopathological examination. In vesiculobullous lesions, two biopsies were obtained in each case. One biopsy was fixed in 10% buffered formaldehyde (pH 7.2-7.4), paraffin embedded and the histological sections cut and stained with hematoxylin and eosin method for analysis of basic histomorphological spectrum. Second biopsy was obtained in normal saline and processed for direct immunofluorescence. In noduloulcerative lesions, sections were routinely stained with hematoxylin and eosin (H and E) and special stains (PAS, Ziehl Neelsen stain, GMS etc.) employed wherever required. Immunohistochemistry was done wherever necessary.

Clinicocytological evaluation was done and results were correlated with histopathological findings. However, in case of vesiculobullous lesions, immunofluorescence findings were also correlated for further confirmation of the diagnosis. The results obtained were then analysed for statistical significance by using Fisher exact test. Sensitivity and positive predictive accuracy were calculated to determine the accuracy of results.

## **RESULTS**

A total of 50 cases were studied which were divided into two groups based on the type of lesion.

Group 1: Vesiculobullous lesions

Group 2: Noduloulcerative lesions.

The following observations were made.

Majority of vesiculobullous disorders presented between 21-30 and 41-50 years age group. Comparatively males slightly outnumbered females in this study. Mean age at onset of the disease was 34.2±19.99 years, with 31.25±15.92 years in

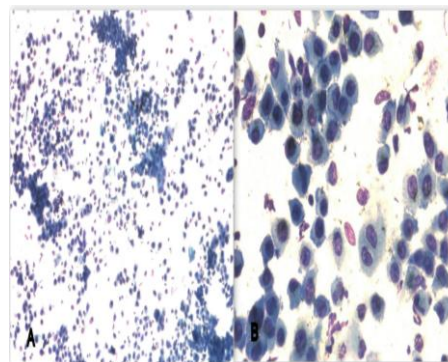
males and  $36.16 \pm 22.77$  years in females. Youngest patient was 3 years and oldest was 68 years in age.

In the present study 20 cases of vesiculobullous disorder were studied, of which pemphigus vulgaris constituted the most common cause (10 out of 20 cases). Pemphigus foliaceus constituted 10% of cases. Bullous pemphigoid, CBDC and viral blisters also shared the same percentage. Pemphigus vulgaris presented most commonly in third decade. Pemphigus foliaceus presented in fifth decade. Bullous pemphigoid presented in sixth decade. Viral blisters were seen in third and seventh decade. Only one case of TEN was included in our study which presented in the seventh decade and a single case of dermatitis herpetiformis seen in the fourth decade.

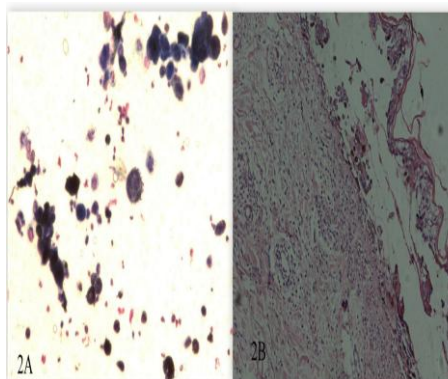
In this study, 6 out of 10 cases of pemphigus vulgaris had only flaccid blisters while 3 cases had both flaccid and tense blisters. Only 1 case presented with tense blisters. Both the cases of pemphigus foliaceus presented with flaccid bullae. Patients of bullous pemphigoid, dermatitis herpetiformis presented with tense blisters while herpes zoster and CBDC patients revealed both flaccid and tense bullae. Base of the blisters varied from erythematous to non erythematous in various disorders. Positive Nikolsky sign was present in all cases of pemphigus group studied (p value 0.000) which was statistically significant.

Acantholytic cells were seen in all cases of pemphigus group [Fig 1a&b] and viral blisters. Presence of acantholytic cells in pemphigus group came out to be statistically significant (p value 0.001). Balloon cells, multinucleated giant cells and ground glass nuclei were seen in cases of herpes zoster studied [Fig 2a&b]. Neutrophils were seen in all cases of pemphigus vulgaris, pemphigus foliaceus, bullous pemphigoid, dermatitis herpetiformis, herpes zoster and also in TEN. Eosinophils were seen in cases of

bullous pemphigoid, dermatitis herpetiformis and few cases of pemphigus group.



**Fig1 (A&B):** Tzanck smear showing acantholytic cells scattered singly & in small groups in a case of Pemphigus Vulgaris; A (Giemsa;200x), B(Giemsa; 400x).



**Fig 2 A :** Tzanck smear showing multinucleated giant cell along with acantholytic cells & few inflammatory cells in Herpes Zoster (Giemsa ;200x); **2 B:** Histopathology showing an intraepidermal bulla filled with acantholytic cells (H&E;200X).

In all cases of pemphigus vulgaris studied, bullae were observed at suprabasal level. Pemphigus foliaceus showed sub corneal blisters. Subepidermal blisters were seen in cases of bullous pemphigoid, CBDC, dermatitis herpetiformis and also in toxic epidermal necrolysis. Blister at intraepidermal level was seen in case of herpes zoster.

Out of 20 cases of vesiculobullous disorders, direct immunofluorescence was done in 17 (85%) cases and was positive in 14 (82.35%) cases. Negative results on immunofluorescence were seen in 3 cases (17.65%). In rest of the 3 cases,

immunofluorescence could not be done due to non availability of the second biopsy.

Intercellular deposition of IgG in a fish net pattern was seen in 8 out of 10 cases of pemphigus vulgaris and both cases of pemphigus foliaceus on direct immunofluorescence. Ig A deposition was seen in basement membrane zone in 2 cases of CBDC and 1 case of dermatitis herpetiformis. One of the two cases of bullous pemphigoid showed IgG and C3 deposition in basement membrane zone.

Out of ten clinically diagnosed cases of pemphigus vulgaris, nine were diagnosed as pemphigus vulgaris and one as pemphigus foliaceus on histopathology. In two cases with clinical suspicion of pemphigus vulgaris, pemphigus foliaceus and bullous impetigo; one was proven on histopathology as pemphigus vulgaris and other as pemphigus foliaceus. Clinical diagnosis matched with the histopathological diagnosis in cases of bullous pemphigoid, CBDC, dermatitis herpetiformis, herpes zoster and toxic epidermal necrolysis

There was 100% cytohistopathological correlation in pemphigus group and in viral blisters which was statistically significant (p value 0.000). Single case of toxic epidermal necrolysis revealed the presence of necrotic keratinocytes, fibroblasts and inflammatory cells, clinching the diagnosis in it's favour. In other vesiculobullous disorders, acantholytic cells were not observed but only inflammatory cells were seen which could not provide a specific diagnosis.

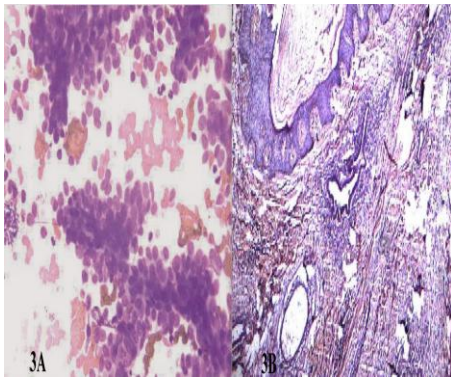
Most of the patients presenting with noduloulcerative lesions were in fifth decade. Mean age of presentation was  $46 \pm 20.52$  years. Youngest patient was five years old female and oldest patient was 80 years male in the study group. Next common age group within this category was in sixth to seventh decade. Out of 30 patients seen, 18 were males and 12 females with a male:

female ratio of 3:2. Males outnumber the females in this study group.

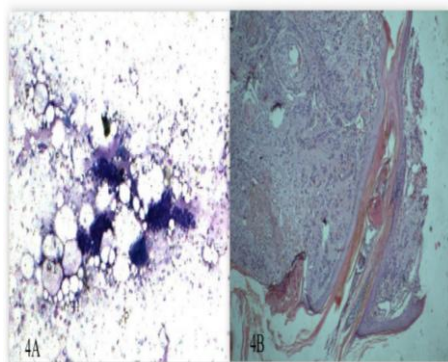
In this study group 29 samples were considered adequate for evaluation, out of which 9 (31.03 %) belonged to non neoplastic category and 20 (68.96%) cases were diagnosed neoplastic lesions on cytology. In neoplastic lesions 6 (20.68%) were benign and 14 (48.27%) were malignant.

Out of 14 cases clinically diagnosed as malignancy, squamous cell carcinoma was diagnosed in 8 cases, malignant small round cell tumor in one and metastatic deposits from renal cell carcinoma in another. Other 4 cases were diagnosed as pilomatricoma, benign skin adnexal tumor, epidermal cyst and endometriosis respectively [Fig3,4]. Two cases with clinical suspicion of basal cell carcinoma, keratocanthoma or dermatofibroma were diagnosed as basal cell carcinoma in one and benign skin adnexal tumor in another. Two cases clinically diagnosed as neurofibroma were correctly interpreted on cytology as neurofibroma [Fig 5] and neurilemmoma. Three cases diagnosed as sebaceous cyst clinically were cytologically proven as epidermal cyst, however one case diagnosed as lipoma came out to be epidermal cyst on FNAC. Scar endometriosis was correctly diagnosed on cytology. One case with clinical differentials of TVC/LV/ chromoblastomycosis was diagnosed as having granulomatous inflammation on cytology. Two cases with an indefinite diagnosis were diagnosed as neurofibroma and keloid on cytopathology. One case, however could not be diagnosed on cytology as aspiration yielded blood only.

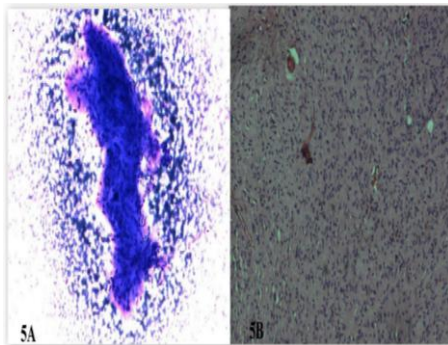




**Fig 3 A:** FNAC smear showing groups of glandular cells along with haemosiderin laden macrophages in cutaneous endometriosis (Leishman; 400x). **B:** Histopathology showing endometrial glands along with stroma lying beneath the epidermis (H&E; 200x).



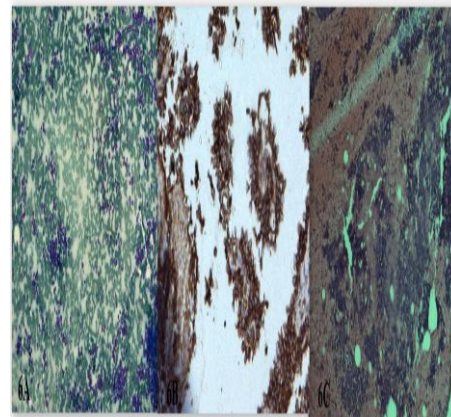
**Fig 4 A:** FNAC smears showing cohesive clusters of benign epithelial cells in a haemorrhagic background in benign skin adnexal tumor (Leishman; 200x). **B:** Photomicrograph of the same case showing Nodular hidradenoma on histology (H&E; 200X).



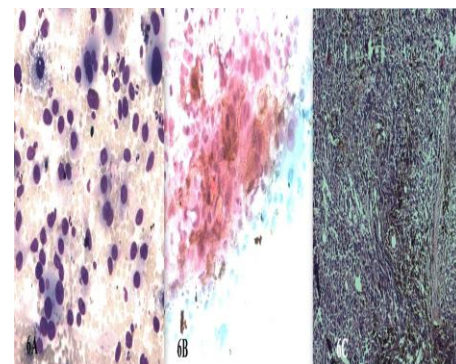
**Fig 5 A:** FNAC smear showing spindled palisading nuclei in a fibrillar background in neurofibroma (Leishman; 200x). **B:** Histopathology showing oval to spindle shaped wavy nuclei in fibrillary background (H&E; 200X).

Cytopathological and histopathological correlation was available in 27 cases. One case considered inadequate for opinion revealed only periadnexal chronic inflammatory infiltrate on histopathology done later. In case of keloid

and metastatic deposits from renal cell carcinoma, histopathological correlation was not available. One case of benign spindle cell lesion was diagnosed as neurofibroma on histopathology. Benign neurogenic/fibromatous lesion was correctly diagnosed as neurofibroma on histopathology. Neurilemmoma diagnosed on cytology was also confirmed histologically. Two cases diagnosed as benign skin adnexal tumor were diagnosed as nodular hidradenoma on histopathology [Fig4]. All the malignant lesions were confirmed as same on histopathology [Fig6,7].



**Fig 6 A:** FNAC smear showing poorly cohesive round or oval cells with high N/C ratio in malignant small round cell tumour (Leishman; 100X); **(B&C):** Cell block from the same case showing PNET with CD99 positivity in tumour cells.



**Fig 7 (A & B):** FNAC smears A: showing pleomorphic malignant cells (Leishman; 400X), B: intracytoplasmic melanin pigment (Pap; 400x) in Malignant Melanoma. **C:** Histopathology of same case showing groups and fascicles of malignant cells containing intracytoplasmic melanin pigment in some (H&E; 200X).

Out of 16 non malignant cases, cytohistopathological correlation was available in 14 cases. In all non malignant

cases, 100% positive correlation was seen. In 14 cases of malignancy diagnosed cytologically, one case of metastatic deposits was not evaluated histologically as it was known case of renal cell carcinoma. Rest of 13 cases were confirmed on histopathology. The cytohistopathological correlation was found to be statistically significant with a p value of 0.000. Cytology was found to have sensitivity of 100% and positive predictive value of 100% in discriminating malignant lesions from the non-malignant ones.

The sensitivity of cytology was 89.36% in diagnosing vesiculobullous and

noduloulcerative lesions of skin. In case of noduloulcerative lesions, exact typing could not be done on cytology in certain cases but a distinction between malignant and non malignant cases could clearly be made.

**Table 1: Cytohistopathological Correlation In Vesiculobullous Lesions**

Cytology diagnosis	Histopathologic diagnosis			
	Pemphigus group	Viral blisters	TEN	Others(BP, DH,CBDC)
Pemphigus	12	00	00	00
Viral blister	00	02	00	00
TEN	00	00	01	00
Non diagnostic	00	00	00	05
Total	12	02	01	05

TEN: Toxic epidermal necrolysis, BP: Bullous Pemphigoid, DH: Dermatitis Herpetiformis, CBDC: Chronic Bullous Disease of Childhood.

**Table 2: Correlation of Type Specific Cytological And Final Histopathological Diagnosis (N=30)**

Sr. No.	Cytopathological diagnosis	No. of cases	Final histopathological diagnosis	No. of cases
1	Cyst of developmental origin	5	Epidermal cyst	4
			Dermoid cyst	1
2	Granulomatous inflammation	1	TVC over LV	1
3	endometriosis	2	endometriosis	2
4	Keloid/ hypertrophic scar	1	-	-
5	Benign spindle cell lesion/tumor	2	neurofibroma	2
6	neurilemmoma	1	neurilemmoma	1
7	skin adnexal tumor	2	Nodular hidradenoma	2
8	pilomatricoma	1	pilomatricoma	1
9	Squamous cell carcinoma	8	Squamous cell carcinoma	8
10	Basal cell carcinoma	2	Basal cell carcinoma	2
11	Malignant melanoma	2	Malignant melanoma	2
12	Malignant small round cell tumor	1	PNET	1
13	Metastatic deposits from RCC	1	-	-
14	Inadequate	1	Periadnexal chronic inflammatory infiltrate	1

**Table 3: Frequency, Age Group And Sex Ratio In Pemphigus Vulgaris**

No.	Features	Fernandez J et al [7] (n=100)	Micali G et al [8] (n=84)	Arya SR et al [9] n=(70)	Nanda A et al [10] (n=128)	Present study (n=20)
1	No. of cases	69(69%)	63(75%)	43(61.4%)	48(80%)	10(50%)
2	Age(years)	22-60	23-87	21-60	-	0-50
3	M:F ratio	1.2:1	1.2:2	1.4:1	1.4:1	1:1.5

## DISCUSSION

Skin diseases are multiple in etiology and clinical appearance. Cytopathology of skin has been documented to be useful in the diagnosis of several skin lesions. Despite the exponential growth and interest in dermatopathology over the years and the fact that the skin is the largest desquamating organ in the body, interest in cutaneous cytology has been limited. Although not a substitute for standard histology, in the hands of an experienced dermatologist Tzanck smears can aid in establishing the

clinical diagnosis with ease and rapidity and can serve as an adjunct to routine histologic study. The technique is cheap, easy to perform and does not cause any discomfort to the patient. [4]

Although cytology should be viewed as a screening tool, many reactions can be classified as inflammatory, hyperplastic or neoplastic. For neoplastic processes, an experienced cytologist can definitively diagnose several specific neoplasms, make a tentative diagnosis of neoplasia for many types of tumours, identify sites of tumour

metastasis and monitor tumour regrowth following anticancer therapy. Information gained from cytology may be useful in establishing a diagnosis, determining a prognosis and formulating a diagnostic or therapeutic plan.

In the present study, an attempt has been made to study clinical, cytological and histopathological features of various vesiculobullous and noduloulcerative lesions of skin and further evaluate the efficacy of cytology in early diagnosis of these lesions

### **Vesiculobullous Lesions**

Most of the patients were seen in the age group of 21 to 40 years. Pemphigus group constituted the most common lesion (12/20) in our study. Similar results were seen by Fernandez et al, [7] Micali et al, [8] Arya et al [9] and Nanda et al [10] as shown in the Table 3. Tzanck smear performed in all 12 patients of pemphigus showed acantholytic cells mixed with a variable number of inflammatory cells which were seen scattered singly or in small groups. On the basis of cytological findings, a diagnosis of pemphigus could be made but further subtyping was not possible. However, as reported by Gupta et al [4] the acantholytic cells in pemphigus foliaceus and pemphigus erythematosus often have a hyalinized cytoplasm in contrast to pemphigus vulgaris, that corresponds to the dyskeratosis seen in tissue sections. Further studies need to be done for evaluation of these features and whether these could be helpful in further typing of pemphigus lesions.

In present study immunofluorescence was done in 17 out of 20 cases. The overall positivity rate of DIF in our study (82.35%) was comparable with the study of Kabir et al [5] (88.23%). Histopathology along with immunofluorescence study were confirmative in these cases. Inappropriate sampling could be the cause of false negativity on DIF. Biopsy taken from the perilesional skin shows characteristic DIF

pattern of pemphigus vulgaris whereas if the biopsy has been taken from the lesional skin, DIF often yields misleading results because of secondary changes. [6]

In all the cases of vesiculobullous lesions studied, tzanck smear served to be of prime importance in the diagnosis of pemphigus group and in case of viral blisters due to herpes zoster. In other cases it helped in ruling out the above mentioned diagnosis and provided a clue for further diagnosis

Tzanck smear has therefore been evaluated in our study as a rapid, inexpensive tool which can be of great value to the dermatologist as a handy, outpatient procedure, saving time and money of the patient for early diagnosis of certain vesiculobullous lesions. Other diseases where tzanck are reported to be of diagnostic value are bullous impetigo, hand, foot and mouth disease, candidiasis, contact dermatitis and Hailey-Hailey disease. However, these patients were not included in our study group. Thus, the number of skin diseases diagnosed on cytology can be relatively increased if tzanck is routinely employed in daily dermatology practice. Infact, the test can be very useful for the diagnosis of pemphigus vulgaris, especially in early stages where biopsy is discomfoting and of little value in arriving at diagnosis.

### **Cytological Diagnosis in Noduloulcerative Lesions**

Though cytological examination is helpful in the rapid confirmation of malignant cutaneous tumors, it does not give much information about the tumor patterns or subtypes which can be of prognostic significance and play an important role in further therapeutic planning. However, it can reliably distinguish between benign and malignant cases which can be of great help to the clinician in deciding further course of management. Further evaluation is thus



needed to broaden the horizons of cytopathology as a diagnostic tool.

Forty seven out of fifty cases of vesiculobullous and noduloulcerative skin lesions were available for cytohistopathological correlation in this study. An accurate diagnosis on cytopathology could be made in 42 out of 47 skin lesions with a sensitivity of 89.36% and a positive predictive value of 100%. Our results are comparable with study done by Sabir et al [1] showing sensitivity of 91.7% in cytological diagnosis of skin lesions.

Thus cytology has been proven as a reliable and informative tool in the diagnosis of various vesiculobullous and noduloulcerative lesions of skin. In comparison to biopsy techniques; it is a non traumatic, inexpensive, safe, well tolerated and a rapid diagnostic tool.

## CONCLUSION

To conclude tzanck smear is a rapid , inexpensive and a safe tool for the early diagnosis of vesiculobullous lesions especially in pemphigus group and viral blisters. Although, not a substitute for standard histopathology, it can serve as a useful adjunct in the diagnosis of certain vesiculobullous disorders. Cytology can be used to differentiate inflammatory lesions from neoplastic, classify neoplastic as benign or malignant, definitively diagnose several specific neoplasms and make a tentative diagnosis in others. As technology continues to advance, cytology is expected to offer much more in diagnosis of various skin lesions. Further studies are thereby

warranted to elucidate the hidden potentials of cytology

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