Role of Imprint/Exfoliative Cytology in Ulcerated Skin Neoplasms

Vishnu Prasad Nelamangala Ramakrishnaiah ·
Ravindra Babu · Dinker Pai · Surendra Kumar Verma

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Abstract Imprint cytology is a method of studying cells by taking an imprint from the cut surface of a wedge biopsy specimen or from the resected margins of a surgical specimen. It is rapid, simple and fairly accurate. Exfoliative cytology is an offshoot from the imprint cytology where in cells obtained from the surface of ulcers, either by scrape or brush, are analyzed for the presence of malignant cells. We undertook this study to see the role of imprint/exfoliative cytology in the diagnosis of ulcerated skin neoplasm and to check the adequacy of resected margins intra-operatively. This was a prospective investigative study conducted from September 2003 to July 2005. All patients presenting to surgical clinic with ulcerated skin and soft tissue tumours were included in the study. A wedge biopsy obtained from the ulcer and imprint smears were taken from the cut surface. Exfoliative cytology was analyzed from the surface smears. Wedge biopsy specimen was sent for histopathological (HPE) examination. The cytology and HPE were analyzed by a separate pathologist. Imprint cytology was also used to check the adequacy of resected margins in case of wide excision. This was compared with final HPE. Total of 107 patients was included in the present study and 474 imprint smears were done, with an average of 4.43 slides per lesion. Out of 59 wide excision samples, 132 imprint smears were prepared for assessing resected margins accounting for an average of 2.24 slides per each excised lesion. On combining imprint cytology with exfoliative cytology the overall sensitivity, specificity and positive predictive value were 90.38 %, 100 % and 90.38 %

respectively. Only one out of 59 cases had a positive resected margin which was not picked by imprint cytology. Imprint cytology can be used for rapid and accurate diagnosis of various skin malignancies. It can also be used to check the adequacy of the resected margin intraoperatively.

Keywords Imprint cytology · Squamous cell carcinoma · Basal cell carcinoma

Introduction

Imprint cytology is a method of studying cells by taking imprint from the cut surface of a wedge biopsy specimen or from the resected margins of a surgical specimen. It is a rapid, simple and fairly accurate method of analyzing the margin of a resected specimen for the presence of malignant cells [1]. Exfoliative cytology is an offshoot from the imprint cytology where in, the cells obtained from the surface of ulcers either by scrape or brush is analyzed for the presence of malignant cells. Some use swab stick to obtain the exfoliated epidermal cells, which are then smeared onto glass slides, fixed and stained. Both the methods of cytology have been used successfully for diagnosing and classifying various tumours, by various authors [1–3].

Various authors have successfully used imprint cytology to diagnose skin malignancies [2, 3], upper gastrointestinal malignancies [4]. Imprint cytology of sentinel lymph node, in carcinoma breast [5] and malignant melanoma [6], is useful in on-table decision making. Imprint cytology has reduced the role of frozen-section in the intraoperative assessment of adequacy of resected margins [7]. One of the prerequisites for successful impression smears is that the cells of the lesion should exfoliate easily. This is especially true for the epithelial tumours. Cells from mesenchymal tissues typically do not exfoliate well, and impression smears will usually be cell-

V. P. N. Ramakrishnaiah (✉) · R. Babu · D. Pai
Department of Surgery, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry 605006, India
e-mail: vprasad285@gmail.com

S. K. Verma
Department of Pathology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India

poor. As a rule, if a diagnostic slide cannot be produced by aspiration from a tissue, it is unlikely that an impression smear will be of good quality. Ulcerated skin malignancies exfoliate easily as they are epithelial tumours. Hence they can be easily imprinted for a quick cytological examination. They require rapid diagnosis and early treatment. There have been few studies that have evaluated the role of imprint cytology in the management of these lesions. Hence this study was done to see the role of imprint cytology in the rapid diagnosis of ulcerated skin neoplasms and to check the adequacy of resected margins intra-operatively.

Methods

This is a prospective, hospital based study, conducted over a period of 22 months, from September 2003 to July 2005. All patients with ulcerated neoplasm of the skin and ulcerated soft tissue tumours, attending the out-patient clinic of department of surgery, were included in the study. Patients with ulcerated neoplasms of the mucosal surfaces like carcinoma tongue and cheek were excluded from the study. A detailed history was taken and clinical examination was done. Imprint smears were taken from the surface of the ulcer. A wedge biopsy was obtained from the ulcer edge. Imprint smears were made from the cut surface of the wedge biopsy specimen. The specimen was kept in 10 % formalin and sent for histopathology. All the imprints were made using the vertical squash technique [8]. A total of 4–6 slides were taken from each patient depending on the surface area that need to be covered. Exfoliative cytology was analyzed from the surface smears.

Imprint smears were stained with May-Grunwald Giemsa stain for the cytoplasmic features and Papanicolaou stain for studying the nuclear features, as per the standard procedure. The wedge biopsy specimen was sent for histopathological examination to confirm the diagnosis. The cytology smears and histopathology slides were analyzed by separate pathologists. The cytologist was blinded to the clinical as well as the histopathological diagnosis. In all the cases the definitive management was excisional surgery, either wide local excision or amputation. Those cases that underwent wide local excision were included for checking the adequacy of resected margins by imprint cytology.

The resected margins of the surgical specimen were imprinted to obtain cytology smears. A total of 2–4 slides were prepared from each case depending on the surface area to be covered. The results of the imprint smear cytology were compared with those of histopathology. The sensitivity, specificity and the predictive values of imprint cytology and exfoliative cytology have been calculated using the final histopathology as the gold standard.

Results

The study included a total of 107 patients with ulcerated skin or soft tissue tumours, who attended the out-patient clinic of department of surgery, between September 2003 and July 2005. There were 86 males and 21 females with age ranging from 24 to 78 years, with a mean age of 51.1 ± 12.1 years. Most of the patients were in 50–59 years age group (Fig. 1). A total of 474 imprint smears were made from 107 patients. An average of 4.43 slides for each ulcerated lesion. For the 59 cases of resected margin assessment group, 132 imprint smears were prepared. An average of 2.24 slides was made for each excised lesion.

The distribution of various lesions is shown in Fig. 2. Squamous cell carcinoma (Fig. 3) was the most commonly encountered malignant lesion, comprising 86 cases. Others included 12 malignant melanomas (Fig. 4), three basal cell carcinomas, two ulcerated breast carcinomas and one ulcerated soft tissue tumour. There were three cases of chronic non-healing ulcers, which clinically looked malignant but both imprint and histopathology diagnosed otherwise. These have been included in the results but excluded in the final analysis. Site wise distribution of squamous cell carcinomas include, 34 penile carcinomas, 36 in the lower limbs, seven in upper limbs, two in scalp, two in the anterior neck, five over the sacrum, gluteal and chest wall regions.

There were 104 malignancies and three chronic non-healing ulcers diagnosed by histopathology. Among the 104 malignancies, imprint cytology was positive in 93 cases and exfoliative cytology in 76 cases. Both were positive in 94 cases. There was only one patient in whom imprint cytology was negative with a positive exfoliative cytology. On comparing exfoliative cytology with histopathology we found that exfoliative cytology had sensitivity of 70.93 %, specificity of 100 % and a positive predictive value of 70.93 % (Table 1). On comparing imprint cytology with histopathology, we found that imprint cytology had sensitivity of 87.21 %, specificity of 100 % and a positive predictive value of 87.21 % (Table 2). Among the 86 squamous cell carcinomas 75 cases were diagnosed by imprint smears and 61 by exfoliative cytology. Only one squamous cell carcinoma had a positive

Fig. 1 Age distribution of patients

Fig. 2 Distribution of lesions encountered in the study

exfoliative cytology with negative imprint cytology. On combining imprint cytology and exfoliative cytology the overall sensitivity, specificity and positive predictive value were 90.38 %, 100 % and 90.38 % respectively. (Table 3) All the melanomas, ulcerated soft tissue tumours and ulcerated breast carcinomas were diagnosed correctly by both imprint and exfoliative smears. The sensitivity, specificity and positive predictive value of imprint cytology for the diagnosis of melanomas and basal cell carcinomas were 100 %, 100 % and 100 % respectively. All the three basal cell carcinomas were accurately diagnosed by imprint cytology but exfoliative cytology was negative in all these three cases.

There were total of ten false negative cases, all of which were squamous cell carcinomas. There were no false positive cases. In addition, grading of squamous cell carcinomas was possible. The degree of differentiation was clearly made out on the cytology smears. Out of the 86 squamous cell carcinomas, 75 were well differentiated, nine were moderately differentiated tumours and two were poorly differentiated. These were accurately graded by imprint cytology also. Most of the skin malignancies could be accurately diagnosed by imprint cytology. In contrast, though malignancy could be diagnosed in the ulcerated soft tissue tumour (malignant fibrous

histiocytoma) and breast carcinomas, the type of malignancy could not be determined. The single soft tissue tumour that was included turned out to be malignant fibrous histiocytoma which was of high grade. The grade and soft tissue origin was accurately diagnosed by cytology but the type of malignancy could not be made out.

Out of the 104 malignancies a total of 59 cases were considered for the assessment of resected margins. These were the cases that underwent wide local resection. Out of these one had positive margins by histopathology which was not picked up on imprint cytology. There were no false positive results on margins reported by imprint cytology technique (Table 4). The imprint cytology reports were available within 30–45 min for all the cases but it took 5–7 days to obtain the histopathology report.

Discussion

Imprint/exfoliative cytology is an easy procedure that does not require anaesthesia and saves time and provides a rapid diagnosis in various tumoral lesions of the body [9]. In our study most commonly encountered skin malignancy was squamous cell carcinoma; next in frequency was malignant melanoma.

Fig. 3 Melanoma on imprint cytology-Papanicolaou stain **Fig. 4** SCC on exfoliative cytology: Giemsa stain

Table 1 Comparison of exfoliative cytology with histology

Type of malignancy	Histopathology positive cases	Positive exfoliative cytology	Sensitivity (%)	Specificity (%)
Squamous cell carcinoma	86	61	70.93	100
Basal cell carcinoma	3	0	0	0
Melanoma	12	12	100	100
Ulcerated breast carcinoma	2	2	100	100
Ulcerated soft tissue tumour	1	1	100	100
Grand total	104	76	72.11	100

Similar studies done by Aryya et al [3], Vega-Memije et al [2] had squamous cell carcinoma as the commonest skin malignancy encountered. Unlike our study, Brown et al [10], Barton et al [11] studied basal cell carcinomas exclusively. Among squamous cell carcinomas majority were located in lower extremity and penis. Aryya et al [3] and Vega-Memije et al [2] predominantly encountered extremity lesions and few penile carcinomas.

We found that imprint cytology was very useful in rapid accurate diagnosis of skin malignancies. The sensitivity and specificity of imprint cytology were 89.42 % and 100 % respectively. All the malignant melanomas and basal cell carcinomas and 87.21 % of squamous cell carcinomas were successfully identified. The combined (imprint cytology and exfoliative cytology) sensitivity, specificity and positive predictive values were 90.38 %, 100 % and 90.38 % respectively. Similar results have been reported by several authors. Brown et al [10] successfully identified all the squamous cell carcinomas and malignant melanomas and 95 % of basal cell carcinomas using imprint cytology when compared with histopathology. Aryya et al [3] found 100 % correlation between

Table 2 Comparison of imprint cytology with histology

Type of malignancy	Histopathology positive cases	Positive imprint cytology	Sensitivity (%)	Specificity (%)
Squamous cell carcinoma	86	75	87.21	100
Basal cell carcinoma	3	3	100	100
Melanoma	12	12	100	100
Ulcerated breast carcinoma	2	2	100	100
Ulcerated soft tissue tumour	1	1	100	100
Grand total	104	93	89.42	100

Table 3 Comparison of imprint cytology with exfoliative cytology in the diagnosis of squamous cell carcinoma

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)
Imprint cytology	87.21	100	87.21
Exfoliative cytology	70.93	100	70.93
Combined	90.38	100	90.38

imprint cytology and histopathology in the diagnosis of skin neoplasms. Vega-Memije et al [2] found that imprint cytology had a 100 % sensitivity and specificity for the diagnosis of squamous cell and basal cell carcinomas. Barton et al [11] found the sensitivity and predictive accuracy of imprint cytology in the diagnosis of basal cell carcinomas to be 92 % and 75 % respectively. They included benign lesions along with basal cell carcinomas in their study. In this study malignant lesions alone have been included in the statistical analysis.

When we compared the results of imprint cytology with the exfoliative cytology, Imprint smears were more useful and had a higher sensitivity and accuracy. There was a single squamous cell carcinoma that had a positive exfoliative cytology and a negative imprint smear report. Exfoliative cytology was not useful in the diagnosis of basal cell carcinomas. None of the three basal cell carcinomas could be identified with exfoliative cytology. Imprint cytology was more useful in that situation. This is probably because of the dry nature of all the three basal cell carcinomas that were encountered in this study.

There was nearly 100 % correlation between imprint cytology and histopathology in the assessment of adequacy of resected margins. Imprint smears failed to identify the single case that had a positive margin on histopathology. There were no false positive reports on imprint smears. In contrast, Florell et al [12] found that the sensitivity of imprint smear for identifying a positive margin was 50 % compared to 70 % with histopathology with respect to Mohs surgical margins for basal cell carcinomas. We found that imprint smear report was available within 45 min in all the cases compared to histopathology report that took 5 to 7 days. This enabled us to have an early diagnosis and avoid the undue delay in starting treatment for the skin malignancies. The imprint smear reports on the adequacy of resected margins were available to the operating

Table 4 Results of resected margin assessment

Types of malignancy (number of cases)	Positive margin Histopathology	Positive margin Imprint cytology
Squamous cell carcinoma (46)	1	Nil
Basal cell carcinoma (3)	Nil	Nil
Melanoma (10)	Nil	Nil
Total (59)	1	Nil

surgeon during the same time, enabling him to provide oncologically sound excisional surgery. Similar findings were reported by Aryya et al [3], Cubuku et al [4] and Creager et al [13] who found that imprint cytology was able to diagnose malignant lesions rapidly and accurately.

Thus Imprint cytology was technically simple and gave rapid, accurate results. It was very sensitive and 100 % specific for diagnosing skin malignancies. The type of skin malignancy was also diagnosed accurately. The grading of squamous cell carcinomas was possible with imprint smear technique and it correlated well with that of histopathology. Malignancies that caused skin ulceration but not arising from it could also be diagnosed. However the type of malignancy could not be diagnosed with imprint cytology. Exfoliative cytology was less sensitive but 100 % specific for diagnosing skin malignancies. It was not useful for the diagnosis of basal cell carcinomas probably because these tumours were dry lesions and did not exfoliate well. Our results were comparable to those reported by most of the authors mentioned in the review of literature.

Imprint smear technique was very useful in intra-operative assessment of resected margins. Though there was one false negative margin there were no false positive margins. Moreover the reports were available in a short time and operating surgeon could make on-table decisions regarding the adequacy of resected margins. He could proceed with further resection, if positive margins were reported. It can be effectively used to cover large surface areas, which is otherwise not easily done with frozen-section technique.

Conclusions

Considering the simplicity of the procedure, minimal discomfort to the patient and rapidity of results, imprint/exfoliative cytology can be used for accurate diagnosis of various skin malignancies. It can also be used intra-operatively, to check the adequacy of resected margins of these lesions. It helps to initiate early treatment by saving the time of waiting for the definitive histopathology report.

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