Efficacy of Imprint Cytology over Histopathological Sections in Head and Neck Lesions

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ABSTRACT

BACKGROUND: The technique of imprint cytology has provided great importance to cytodiagnosis due to its simple, cost effective, excellent cellular details and rapid results. While diagnosing malignant tumour it plays a significant role in avoiding multiple re-excisions for margin clearance and helps optimize cosmetic results in patients undergoing conservative surgery.

OBJECTIVES OF THE STUDY: 1. To study the efficacy of touch imprint cytology as a diagnostic tool in identifying malignancies. 2. To correlate the diagnosis on touch imprint cytology with that of the histopathology.

METHODOLOGY: A total of 57 surgically resected specimens from Head and neck lesions in DR. B. R. Ambedkar Medical College and hospital from November 2017 to June 2019 were evaluated. Imprint smears of freshly excised specimens before fixation in formalin were made by pressing the cut surface of tissue against the glass slide for suspicious foci. The Imprint slides were fixed in 95% ethyl alcohol and stained as per standard protocol using hematoxylin and eosin (H& E) and papanicolaou (pap) stains. Diagnosis was made on detailed light microscopic examination and clinical information.

RESULTS: The present study is prospective study with a total of 57 cases of head and neck lesions being studied. Predominantly encountered were non- neoplastic lesions of thyroid, followed by cervical lymph node, salivary glands and others. Imprint cytology diagnosis in comparison with HPE diagnosis had sensitivity of 87.5% with 100% specificity positive predictive values of 100% and negative predictive value of 95.35%. Overall diagnostic accuracy of detecting type of lesion was 96.49%. Kappa agreement between imprint cytology and HPE was 0.9097.

CONCLUSION: The use of smear technique in intraoperative diagnosis provides a rapid and efficient means of pathological assessment. It is capable of obtaining a high degree of accuracy and hence, its use is highly recommended routinely.

KEYWORDS: Imprint cytology, Thyroid neoplasm's, Salivary neoplasm's, Histopathology, Accuracy, Lymphadenopathies, Benign, Malignancy.

INTRODUCTION

The scope of imprint cytodiagnosis as a simple, rapid and reliable diagnostic method was elucidated as early as 1927 by Dudgeon and Patrick through their pioneering work and still persists to be explored even in the modern era of diagnostic medical practice. Recently touch imprint cytology is being utilised as an adjunct to assess the adequacy of the sample obtained by Ultra Sound or CT guided biopsies and is found to be very useful in reducing the number of passes a radiologist may have to perform on a particular patient.[1] With the comparable sensitivity and specificity to Frozen Section, it is quick, reliable and does not require elaborate apparatus, hence cost effective in providing excellent cellular details.[2]

With a mean accuracy of 91%, the literature suggests that in head and neck lesions, intra-operative touch preparation could potentially evaluate the margins, differentiate benign and malignant thyroid lesions, determine the parathyroid tissue, sentinel lymph nodes and basal carcinomas of the skin and assess salivary gland tumors.[2,3,4] This decreases the need for multiple re-excisions and thereby improve cosmoses, even in institutions with limited resources.

Although histopathological appearance of a lesion in any organ is considered to be the final arbiter of its diagnosis, yet the delay involved may at times affect the course of treatment in certain situations.[5] Imprints of freshly resected surgical specimens give an excellent cytomorphology and when used in conjunction with rapid pap staining a fully accurate diagnosis can be offered within minutes, overcoming this delay.[6] However, the limitations like inability to differentiate in-situ carcinoma from invasive carcinoma and provide architectural details still persists.[7]

In an era in which cost containment has become a critical factor this study aims to identify the efficacy of touch imprint cytology as a diagnostic tool in identifying malignancies and correlate the diagnosis with that of the histopathology.[7]

MATERIALS AND METHODS:

The study is a prospective observational study, conducted in the Department of Pathology, B.R Ambedkar medical college, Bengaluru, India for a period of 2 years from November 2017 to June 2019. All freshly received specimens of head and neck surgeries for the above mentioned period constituted the study sample.

The detailed clinical history for each case was obtained. The specimens were thoroughly examined grossly, serially sectioned and 4-5 imprint smears were made under the guidance of the operating surgeon. The excess blood and fluid on the cut surface were blotted using a filter paper and subsequently the slides were gently pressed against the cut surface. This allowed the removal of excess fluid and blood that could obscure the cytological feature and thereby improve the results. Clean grease free glass slides of standard parameters are used. These slides were immediately fixed with 95% of ethyl alcohol for 5-6 seconds and stained with Haematoxylin and Eosin and a few slides with Papanicolaou staining.

The morphology of cells was studied under the low power & high power (10X & 40X) respectively and a cytological diagnosis was made. Later sections from the same specimens underwent tissue processing and H& E staining for the routine histopathological examination and definitive diagnosis, which were correlated with the cytology impression.

STATISTICAL ANALYSIS

was performed using SPSS 22 version software (IBM SPSS Statistics, Somers NY, USA). Continuous data was represented as mean and standard deviation and categorical data was represented in the form of frequencies and proportions. Chi-square test was used as test of significance for qualitative data and the agreement between HPE and Imprint cytology was assessed by using Kappa statistics. p value of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

RESULTS

57 cases of head and neck lesions with majority of subjects (26.3%) in the age group 31 to 40 years (Mean age of 42.4) and male to female ratio of 0.9:1 was a part of the study (Table 1). Site of maximum lesions were thyroid, followed by lymph nodes, salivary gland and adenotonsils (Figure 1). 77.2% patients presented with complaints of diffuse Swelling, 7% with solitary swelling and ulcer respectively, 3.5% with multiple swelling and pre auricular swelling respectively and 1.8% with submandibular swelling, of which 66.8% cases had no significant past history against to which 12.3% had history of fever and 8.8% had previous history of carcinomas.

Grossly the cases had varied presentations (Figure 2). The HPE and imprint cytology diagnosis are listed down in Table 2 & 3 respectively. A significant association among the two diagnoses could be established in the study (Figure 3). Imprint Cytology had Sensitivity of 87.5%, Specificity of 100%, Positive Predictive Value of 100%, Negative Predictive Value had 95.35% and Diagnostic Accuracy of 96.49% and Kappa agreement between Imprint Cytology and HPE was 0.9097 (almost perfect agreement).

TABLE 1: AGE AND SEX DISTRIBUTION OF THE SUBJECTS

		Count	%
Age	<20 years	9	15.8%
	21 to 30 years	8	14.0%
	31 to 40 years	15	26.3%
	41 to 50 years	6	10.5%
	51 to 60 years	9	15.8%
	61 to 70 years	5	8.8%
	>70 years	5	8.8%
	Total	57	100.0%
Sex	Female	30	52.6%
	Male	27	47.4%

TABLE 2: IMPRINT CYTOLOGY DIAGNOSIS DISTRIBUTION AMONG SUBJECTS

			Count	%
Imprint cytology diagnosis	BST, PA	Benign salivary gland tumour, Pleomorphic adenoma	2	3.5%
	CAT	Chronic adenotonsilitis	2	3.5%
	CG	Colloid goiter	8	14.0%
	DC	Dermoid cyst	2	3.5%
	DSL	Dysplastic skin lesion	1	1.8%
	EIC	Epidermal inclusion cyst	2	3.5%
	FA	Follicular adenoma	1	1.8%
	GL	Granulomatous lymphadenitis	8	14.0%
	HL	Hodgkin's Lymphoma	2	3.5%
	HT	Hashimoto's thyroiditis	1	1.8%
	L	Lymphadenitis	2	3.5%
	M	Metastasis	3	5.3%
	M, SO	Metastasis, Squamous cell origin	1	1.8%
	NG	Nodular goiter	2	3.5%
	NS	Nothing significant	1	1.8%
	NT	No tumour	3	5.3%
	PA	Pleomorphic adenoma	4	7.0%
	PMCRO	Possibility of malignancy cannot be ruled out.	1	1.8%
	RC	Retention cyst	1	1.8%
	RL	Reactive lymphadenitis	2	3.5%
	SCC	Squamous cell carcinoma	4	7.0%
	SCD	Squamous cell differentiation	1	1.8%
	SM, SCC	Suspicious of malignancy	2	3.5%
	SM, SO	Suspicious of malignancy, Squamous cell origin	1	1.8%

TABLE 3: HPE DIAGNOSIS DISTRIBUTION AMONG SUBJECTS

			Count	%
HP Diagnosis	AG	Adenomatoid goiter	1	1.8%
	CAT	Chronic adenotonsilitis	2	3.5%
	CG	Colloid goiter	2	3.5%
	CGL	Chronic granulomatous lymphadenitis	1	1.8%
	CNSL	Chronic non specific lymphadenitis	1	1.8%
	DC	Dermoid cyst	1	1.8%
	EIC	Epidermal inclusion cyst	1	1.8%
	GL	Granulomatous lymphadenitis	4	7.1%
	HCG	Hypertrophic colloid goiter	1	1.8%
	HL	Hodgkin's lymphoma	3	5.3%
	HNG	Hypertrophic nodular goiter	1	1.8%
	HT	Hashimoto's thyroiditis	1	1.8%
	L	Lymphadenitis	2	3.5%
	LWD	Leukoplakia without dysplasia	1	1.8%
	MEC	Mucoepidermoid carcinoma	2	3.5%
	MNG	Multinodular goiter	2	3.5%
	MNP	Malignant Neoplastic process	1	1.8%
	MRC	Mucous retention cyst	1	1.8%
	MSO	Metastasis Squamous origin	3	5.3%
	NG	Nodular goiter	4	7.0%
	NGP	Necrotizing granulomatous process	1	1.8%
	NP	Normal parotid	1	1.8%
	NT	No Tumour	4	7.0%
	PA	Pleomorphic adenoma	5	8.8%
	RL	Reactive lymphadenitis	1	1.8%
	SCC	Squamous cell carcinoma	7	12.3%
	TGC	Thyroglossal cyst	1	1.8%
	TL	Tuberculosis Lymphnode	2	3.5%

TABLE 4: ASSOCIATION BETWEEN HPE DIAGNOSIS AND IMPRINT CYTOLOGY DIAGNOSIS

		HPE Diagnosis					
		Malignant		Benign		Total	
		Count	%	Count	%	Count	%
Imprint Cytology diagnosis	Malignant	14	87.5%	0	0.0%	14	24.6%
	Benign	2	12.5%	41	100.0%	43	75.4%
	Total	16	100.0%	41	100.0%	57	100.0%

$\chi^2 = 47.55$, df =1, p <0.001*

In the study among those with malignant lesions in HPE, 87.5% were malignant lesion and 12.5% were benign lesion in Imprint cytology. Among 41 benign lesions in HPE, 100% were benign in Imprint Cytology diagnosis. There was significant association between HPE Diagnosis and Imprint Cytology diagnosis.

TABLE 5: VALIDITY OF IMPRINT CYTOLOGY DIAGNOSIS IN COMPARISON WITH GOLD STANDARD HISTOPATHOLOGY DIAGNOSIS

| Parameter	Estimate	Lower - Upper 95% CIs
Sensitivity	87.5%	63.98, 96.5
Specificity	100%	91.43, 100
Positive Predictive Value	100%	78.47, 100
Negative Predictive Value	95.35%	84.54, 98.72
Diagnostic Accuracy	96.49%	88.08, 99.03
Cohen's kappa (Unweighted)	0.9097	0.6511 - 1.168

Imprint Cytology had Sensitivity of 87.5%, Specificity of 100%, Positive Predictive Value of 100%, Negative Predictive Value had 95.35% and Diagnostic Accuracy of 96.49% and Kappa agreement between Imprint Cytology and HPE was 0.9097 (Almost perfect agreement).

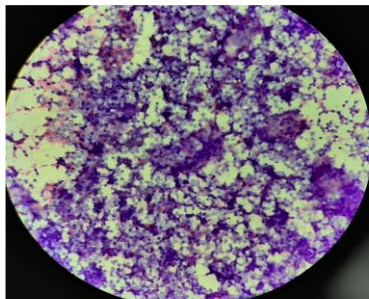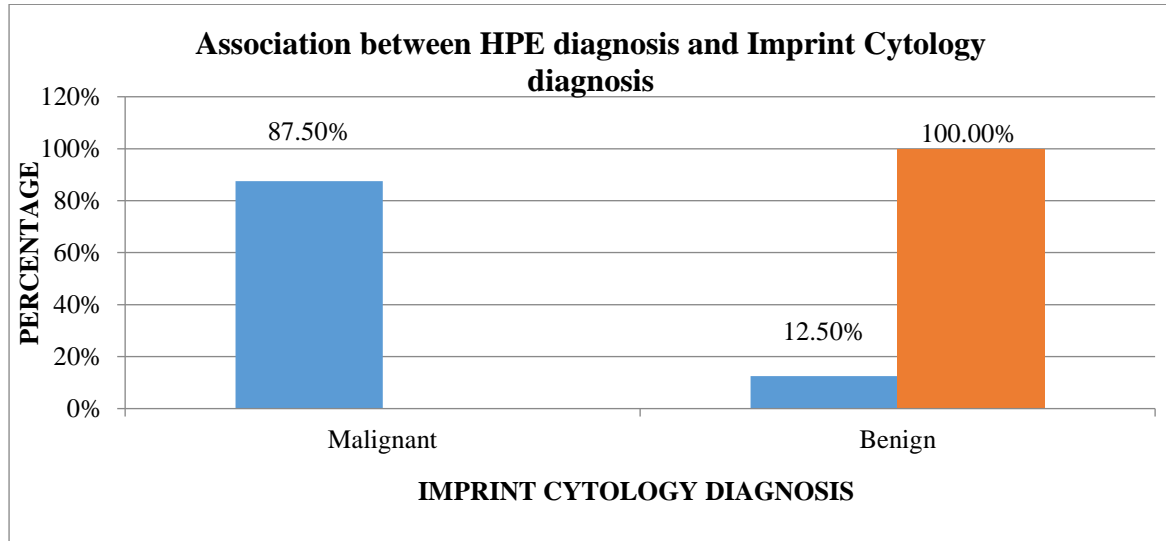**FIGURE 1: SITE OF LESION DISTRIBUTION AMONG SUBJECTS**
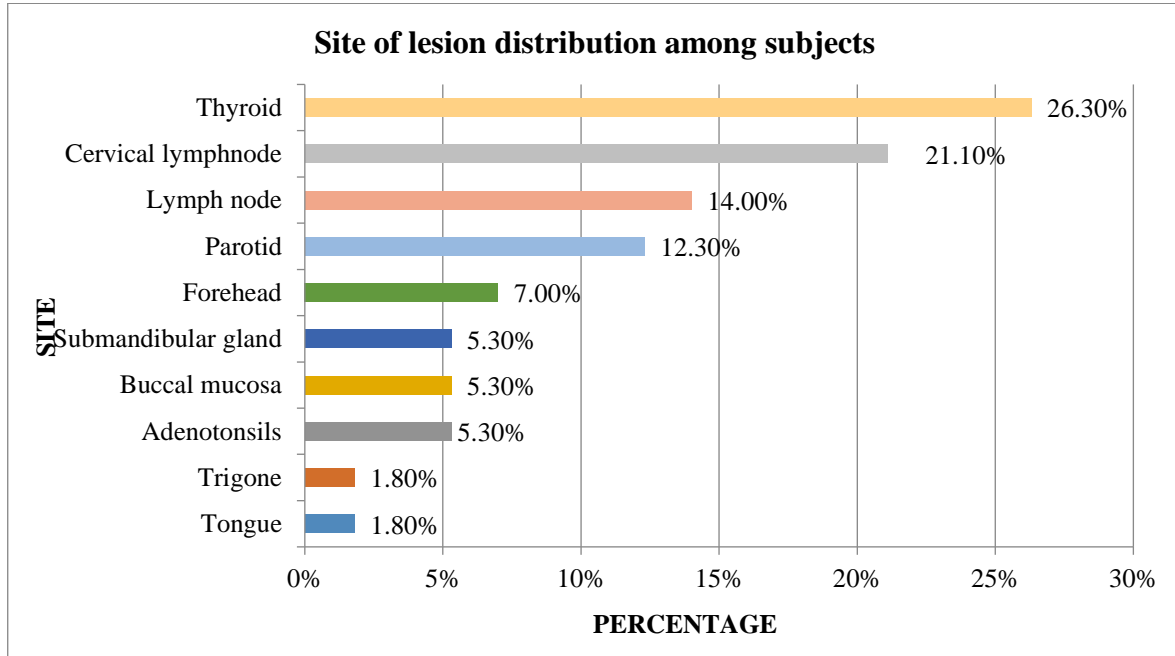
**FIGURE 2: EXTERNAL & CUT SURFACE FINDINGS AMONG THE LESIONS**
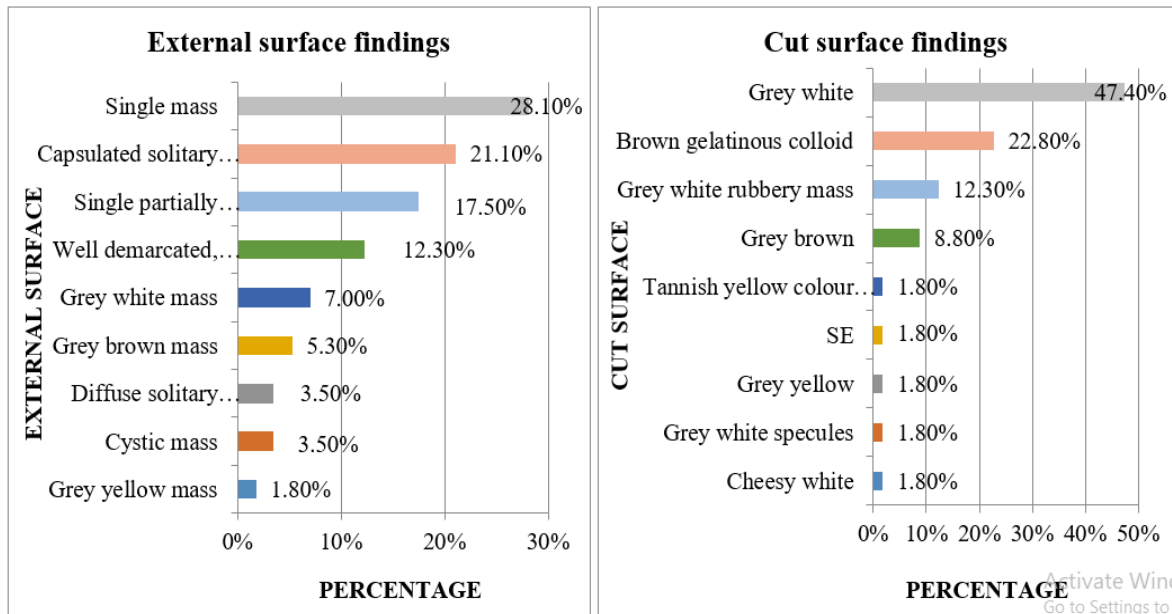
**FIGURE 3: ASSOCIATION BETWEEN HPE DIAGNOSIS AND IMPRINT CYTOLOGY DIAGNOSIS**

Imprint cytology : (10X Low power view,H & E Stain)of Hashimoto's thyroiditis showing hypercellular smear consisting of clusters of thyroid follicle cells along with lymphocytes admixed with in them.

Gross image of Hashiomoto's thyroiditis
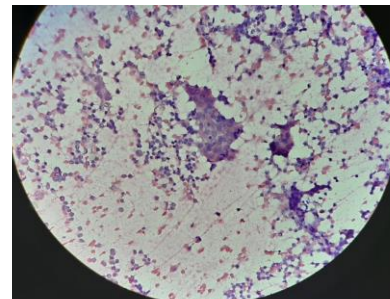
Imprint cytology : (10X Low power view , H & E Stain) showing Hurthle cells admixed with few lymphocytes and scant colloid in hashimoto's thyroiditis.
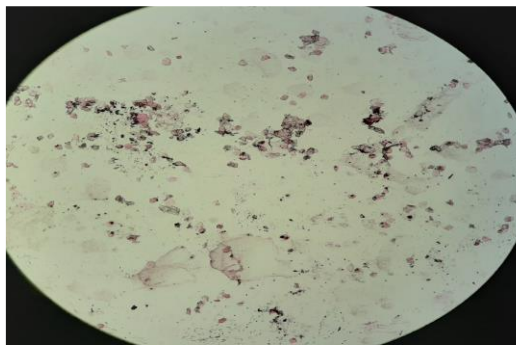
(4X Scanner view, H & E Stain) A case suggestive of Epidermal inclusion cyst showing squamous epithelial cells and keratin flakes admixed in a dirty background .
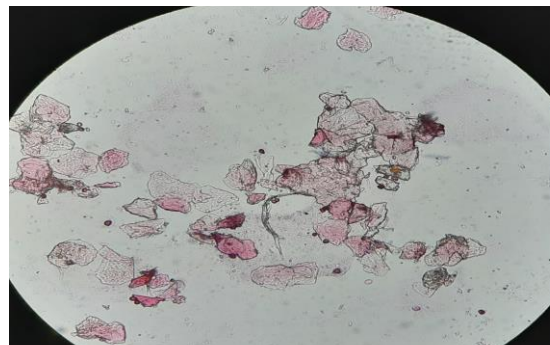
(40X High power view, H & E Stain) A case suggestive of Epidermal inclusion cyst showing Anucleate squamous epithelial cells admixed in a background of dirty cellular debris .

DISCUSSION

The present study was conducted with an aim to evaluate the diagnostic utility of imprint cytology. The benign lesions were seen in comparatively younger age group as compared to malignant lesions. This was statistically significant for the occurrence of benign and malignant lesions. Similar finding was noted in the study conducted by

Orki et al. who observed that mean age for malignant lesions was 54.2 years. [8]

Among the study group, only six (6%) cases had poor cell yield, of which majority were benign lesion. Two were cases of multinodular goiter in hyper involution, which on HPE showed thyroid follicles over distended with colloid and lined by flattened cells and the third case was of nodular sclerosis type Hodgkin's lymphoma. The fourth case of dermoid cyst of forehead showed mainly keratinous material. Thereby the cause of poor cellularity was analysed.

All malignant cytological diagnoses turned out to be malignant on HPE except for two cases which were diagnosed as benign lesions in touch preparations. Though we could diagnose that these were suspicious of malignancy, we were unable to pinpoint the exact nature of tumor in few cases. Liang et al. analysed the discrepancy between results of touch imprint cytology and histopathology and concluded that the two major causes included interpretation error followed by sampling error. The specificity, negative predictive value, and accuracy were 98.7%, 94.6%, and 93.8% respectively in their study.[9]

Nevertheless, the overall accuracy of detecting the type of lesion was 96.4%. The study conducted by Suen et al. showed that imprint technique had a wide application with overall accuracy of 96.3%.[10] Similar was the findings by Lee which included comparison of imprint cytology and HPE diagnosis of 522 tumor cases from various sites, where the total diagnostic accuracy was 92.9%. Overall, the false positive and false negative rates were 0.8% and 4.8%, respectively.[11] Scopa et al. who evaluated tissue imprints in surgical pathology found that intraoperative imprint cytology achieved the accuracy was 97.5% and 91% respectively for benign and malignant lesions with an overall accuracy rate of 94.3%. This study also highlighted that the diagnostic yield, when intraoperative imprint cytology and frozen section were used together was 99%.[12]

The head and neck lesions showed that the overall accuracy in detecting lesion is 96.4%. Various other studies have shown it in the range of 89%–97%.[13-17]

Despite the good cytomorphological details obtained on the smears, certain practical difficulties were faced in the study. Limitations included difficulty in identifying the cell of origin in certain malignant cases, where in correct interpretations wasn't possible on imprints alone. For such lesions close differential diagnosis can be given. Lack of cellular material, over stained and under-stained smears and necrotic material obscuring the cellular details challenged the diagnosis.

Among the thyroid lesions, differentiating follicular adenoma from adenomatoid nodular goitre was almost always challenging. Similar diagnostic difficulties were encountered in 5 cases of lymph node resection specimens, which accounted to 25% of the LRS.

In case one, imprint showed poor cell yield, with dysplastic cells. Considering this, the diagnosis of squamous cell differentiation was entertained. However, this turned out to be metastasis from mucoepidermoid carcinoma. In cases two, the imprint showed poor cell yield, with necrotic material admixed with acute inflammatory cells and few ill formed granulomas. Diagnosis of granulomatous lymphadenitis was considered. Histopathological examination revealed the diagnosis to be nodular sclerosis type of Hodgkin's lymphoma. In case three, imprint smears showed few anaplastic tumour cells, hence probability of metastasis was considered. But, HPE sections showed squamous cell carcinoma. The imprint smears in the fourth case were highly cellular with atypical squamous cells and cytodiagnosis of suspicious of malignancy was given. HPE sections later showed metastasis from squamous cell origin. In case five, imprint cytology diagnosis was in favour of an epidermal inclusion cyst in the presence of keratin flakes, squamous cells and karyorrhectic debris in the background but the

histopathological the final diagnosis was of lymphadenitis.

The overall accuracy in detecting lesions in LRS in our study is 75.8% with sensitivity of 87.5%. Other studies that focussed on lymph node imprint cytology yielded a sensitivity of 64%–93% and accuracy of 86%–98%.[8,18]

Our study could explain the association between imprint and HPE diagnosis. The overall accuracy of touch imprint cytology obtained in our study is 82.5%, in the background of a small sample size. The earlier studies with larger sample size have shown better correlation.[19, 20]

CONCLUSION

Rapid intraoperative evaluation is critical in patient care with respect to decreasing morbidity, cost and overall prognostic outlook. Advantages like better cellular details obtained in touch imprints when used as an adjunct to frozen section can ennoble the role of pathology in intraoperative consultation. Although the purpose of our study is not to give a precise measurement of the diagnostic performance of imprint cytology, the results underscored that the inclusion of cytology in the intra-operative diagnostic procedure enhanced its concordance with the definitive histopathological diagnosis.

Despite the simplicity, speed and excellent cellular detail the technique has still not been fully utilized. Nevertheless, we advocate the consideration of touch imprint cytodiagnosis for the evaluation of cases with nodal metastases as a valuable alternative to frozen section histology in day-to-day practice for better management of patients.

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