Evaluation of Role of Intraoperative Cytology Technique in Diagnosis and Management of Cancer

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Abstract

Context: Intraoperative pathological assessment provides valuable information in a patient’s diagnosis and management. Touch imprint/crush cytology is simple, rapid, and cost-effective. Also, imprint smears give excellent cytomorphology. Aims: To assess the utility and feasibility of intraoperative cytology technique as a rapid and reliable method for diagnosis and to compare sensitivity, specificity and diagnostic accuracy with histopathology. Materials and Methods: Cytology smears were collected intraoperatively from 52 cases of suspected/proven malignancy. From tumors, surgical margins, lymph nodes; crush, imprint, and scrape smears were prepared. Smears were taken from specimens before formalin fixation and stained with rapid ultrafast Papanicolaou stain. The slides were compared with the histopathology report which is the gold standard. Statistical Analysis: Sensitivity, specificity, and diagnostic accuracy were used for statistical analysis. Results: Intraoperative cytology report could be given in time for the surgeon to modify margins of resection and the extent of lymph node dissection. It helps the surgeon to modify surgery. The diagnostic test evaluation showed satisfactory results. Conclusion: Intraoperative imprint/crush cytology is a simple, inexpensive, rapid, accurate cytdiagnostic technique in the diagnosis of cancer where frozen section facilities are not available. It can also be used to assess the clearance of surgical margins.

Keywords: Crush smear, cytodiagnosis, imprint, intraoperative, rapid PAP

INTRODUCTION

The primary purpose of the intraoperative (IO) pathologist consultation is to guide immediate surgical management.[1] Intraoperative consultations are sought by surgeons for various reasons, including diagnosis of a previously undiagnosed lesion (mainly benign versus malignant), assessment of margin status, detection of spread of disease, for example, lymph nodes metastasis and instant evaluation of the adequacy of lesional tissue.[2]

In head and neck tumor resections, surgeons often plan to perform reconstructive surgery immediately after definitive resection, and establishing the presence of negative margins intraoperatively is crucial.[3] Successful reconstruction of the defect is extensively limited by the oncological clearance at margins. The surgeon modifies his surgical plan based on the intraoperative consultation from the pathologist.[4]

Two methods for intraoperative diagnosis commonly in use are imprint cytology and frozen section (FS).[5] An FS, one of the most valuable intraoperative tools for case management involves teamwork among pathologists, pathology trainees, and sometimes pathology assistants.[6] Several factors can influence the accuracy of FS diagnoses, such as tumor size, histologic type, and the pathologist’s experience.[7]

Imprints of freshly resected surgical specimens give excellent cytomorphology and when used in conjunction with rapid pap staining, a fully accurate diagnosis can be offered within minutes.[8] The simplicity, speed, and cost-effectiveness of the technique along with its ability to maximize cell recovery from very small tissue pieces make touch imprint cytology a valuable resource for virtually every aspect of experimental and diagnostic medicine.[9]

Access this article online

Quick Response Code:

Website:
www.jcytol.org

DOI:
10.4103/JOC.JOC_94_19

How to cite this article: Jaiswal YP, Gadkari RU. Evaluation of role of intraoperative cytology technique in diagnosis and management of cancer. J Cytol 2020;37:126-30.

Submitted: 24-Aug-2019; Revised: 02-Oct-2019; Accepted: 14-Apr-2020; Published: 30-Jun-2020

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This study was designed to investigate the accuracy and feasibility of imprint or crush cytology in cancer diagnosis and in resection of margins. The results were compared with corresponding paraffin sections, which is the gold standard.

**Material and Methods**

The study was conducted in the pathology department at a tertiary health care center between August 2015 and September 2017. The study was approved by the ethical committee of the institute. It is a descriptive observational diagnostic test evaluation study. All patients with suspected or proven malignancy in which intraoperative diagnostic aid is sought by clinicians during the study period were included.

Neonates, pregnant females, and HIV patients were excluded. In each case data were collected prospectively as per proforma and complete clinical history, examination, and clinical diagnosis were recorded in a proforma. Results of all preoperative investigations like fine-needle aspiration cytology (FNAC), previous biopsy, CT, MRI, and USG were recorded. Informed consent was obtained from all patients. The morphology of previous tissue or cell sample on FNAC or histopathology was studied prior to intraoperative cytology.

**Collection of specimens**

A total of 52 cases was evaluated. Cytology smears were collected intraoperatively from cases of suspected/or proven malignancy. Gross examination of the lesion was recorded. Glass slide was pre-labeled with patients’ initials, site, and type of specimen. The specimen was cut into two halves. The cut surface was wiped off the excess blood with the filter paper or wet gauze. To prevent contamination, a single surgical blade was used for each specimen. From tumors, surgical margins and lymph nodes; crush, imprint, and scrape smears were prepared depending on the consistency of lesion. Touch imprints were prepared by touching freshly cut, flat surface of the specimen on glass slide gently but with firm pressure twice or thrice. For crush smears the small particles were collected on a glass slide and “crushed” with a second slide. In fibrous or sclerotic lesions, the surface was scraped with the scalpel blade and the collected material was smeared on a slide. A minimum of two smears were prepared for each specimen. These smears were prepared from the already resected specimen and no additional tissue was collected for the purpose of the study. While preparing smears from a lymph node, freshly received lymph node was measured and fibrofatty tissue surrounding the lymph node was removed without disturbing the capsule. Excised lymph nodes were bisected along the long axis. Care was taken to obtain complete cross-sections of the maximum dimension.

In oral carcinoma, the orientation of the resected tumor was done by a surgeon. Margins were identified and labeled as anterior, posterior, medial, lateral, superior, and inferior. The exact number and nomenclature of margins depend on the location of the tumor. The lesions were resected along with 1–2 cm of clinically visible normal tissue. After resection of the lesion margins were excised from the edge of the resected specimen. The cut tissue was held by forceps with the freshly cut flat surface upward. On the other hand, cleaned pre-labeled glass slide was touched lightly onto serial adjacent areas on the cut surface of the tissue. If the touched surface was excessively bloody, the slide was discarded and the procedure was repeated with another slide. Margins that were sent as small bits from deeper tissue were crushed. For crush smears the small particles were collected on a glass slide and “crushed” with a second slide. An average of three to four slides were prepared. All of these smears were fixed in 95% alcohol and stained with rapid ultrafast Papanicolaou (PAP). The total time is taken for smear preparation, staining, and reporting was about 15 min. Smears were reported as positive or negative for malignancy. If the material was inadequate, especially in lymph nodes, it was reported as such. The examination report was conveyed to the operating surgeon. A further line of management was decided on the table based on the intraoperative cytology report. Since histopathology was done in all cases, comparison with this gold standard was done for calculation of sensitivity, specificity and diagnostic accuracy. In nodes, which were positive on cytology but negative on histopathology, more sections were studied.

**Results**

We evaluated 160 specimens from 52 cases as per Table 1. The majority (N = 37) were oral cancers. The rest was as needed by surgeons.

Out of the 160 specimens examined in the study, 58 were positive for malignancy and 102 were negative for malignancy on intraoperative technique. Table 2 shows a comparison of intraoperative cytology with histopathology.

The positive test result was found in 64 samples out of 160 specimens. It was found that the prevalence of the positive test was 40%. The sensitivity and specificity of the

| Table 1: Distribution of cases |
|-------------------------------|
| Organs                   | Tissue diagnosis                  | No. of cases |
| Buccal mucosa            | Squamous cell carcinoma           | 23           |
| Tongue                   | Squamous cell carcinoma           | 10           |
| Larynx                   | Squamous cell carcinoma           | 4            |
| Thyroid                  | Medullary carcinoma               | 1            |
|                         | Lymphoma                          | 1            |
|                         | Anaplastic carcinoma              | 2            |
| Breast                   | Invasive duct carcinoma           | 3            |
|                         | Lobular carcinoma                 | 1            |
| Salivary gland           | Pleomorphic adenoma               | 1            |
|                         | Adenoid cystic carcinoma          | 1            |
| Frontal sinus mass       | Schwannoma                        | 1            |
| Stomach biopsy           | Adenocarcinoma                    | 1            |
| Adrenal mass             | Adrenocortical carcinoma          | 1            |
| Retroperitoneal mass     | Schwannoma                        | 1            |
| Abdominal mass           | Papillary adenocarcinoma          | 1            |
| Total                    |                                  | 52           |
intra-operative technique were 75% and 89.6% respectively and the positive predictive value (PPV) and negative predictive value (NPV) were 82.8% and 84.3%, respectively. The positive likelihood ratio of the intraoperative technique was 7.2 and the negative likelihood ratio was 0.279. The intraoperative imprint technique had a diagnostic accuracy of 83.8%. The area under the ROC curve was 82.3%. Table 3 shows the diagnostic test evaluation for 160 specimens. Margins and Lymph nodes were evaluated separately.

A total of 104 specimens were collected from the margin sites (20 from the anterior margins, 21 from the posterior margins, 23 from the superior margins, 20 from the inferior margins, 11 from the medial margins, 9 from the lateral margins). The sensitivity and specificity of the intraoperative technique for margins were 61.5% and 87.7%, respectively and the positive predictive value and negative predictive value were 75.0% and 79.2%, respectively. The positive likelihood ratio of the intraoperative technique was 5 and the negative likelihood ratio was 0.44. The intraoperative imprint technique had a diagnostic accuracy of 77.8% for margins [Table 4].

The sensitivity and specificity of the intraoperative technique for lymph nodes were 90% and 93.5% respectively and the positive predictive value and negative predictive value were 81.8% and 96.7% respectively. The positive likelihood ratio of the intraoperative technique was 13.9 and the negative likelihood ratio was 0.107 [Table 5].

The intraoperative imprint technique had a diagnostic accuracy of 92.7% for lymph nodes.

The diagnostic accuracy of intraoperative imprint technique and FNAC was compared with gold standard histopathology in 15 different organ specimens. The results are given in Table 6.

The diagnostic accuracy of the imprint technique to differentiate between benign and malignant (for these 15 cases) was 100%. The accuracy of the imprint technique for the typing of malignancy (for these 15 cases) was 86.67%.

**Discussion**

Of the total 160 specimens studied in 52 cases, 41 specimens were imprints of cervical lymph nodes, 104 specimens were crushed smears of margins of squamous cell carcinoma of the oral cavity and 15 specimens were imprinted, crush, and scrape smears of various tumors. 48 specimens were true positive, 86 specimens were true negative, 10 specimens were false positive, 16 specimens were false negative. Among 10 false positives, eight false positives were of margins and two false-positive were of lymph nodes. Of the total 16 false negatives, 15 false-negative specimens were of margins and one false negative specimen of the lymph node.

The diagnostic accuracy of the intraoperative technique for diagnosis of malignancy was 83.8%. The sensitivity and specificity were 75% and 89.6% respectively. In lymph nodes diagnostic accuracy, sensitivity and specificity were 96.7%, 90% and 93.5%, respectively. In margins, the indices were 77.8%, 61.5% and 87.7% respectively. In organs, accuracy for diagnosis of malignancy was 100% and for typing, it was 86.67%.

According to Yadav et al.,[11] the false positive rate in margins was due to the presence of cauterized cells, contamination by tumor cells at margin while manipulation of tissue. The false-negative rate could be due to the failure of tumor cells to adhere to the glass slide. The nature of cells in the head and neck, where large numbers of cases are squamous cell carcinoma, which were well-differentiated, may cause less loss of cell cohesion resulting in low cellularity in margins. It could also be due to the focal extension of the tumor at the margin. Tumors that closely approximate but do not reach the margin were not imprinted or crushed. Other reasons are representative sample might not be obtained while preparing crush smears or insufficient tissue obtained.

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**Table 2: Comparison of intraoperative cytology with histopathology**

| Intraoperative imprint and crush | Histopathology | Total |
|----------------------------------|----------------|-------|
|                                  | Positive | Negative |     |
| Positive                        | 48      | 10       | 58   |
| Negative                        | 16      | 86       | 102  |
| Total                            | 64      | 96       | 160  |

**Table 3: Diagnostic evaluation**

| Parameter                     | Estimate |
|-------------------------------|----------|
| Sensitivity                   | 75%      |
| Specificity                   | 89.6%    |
| Positive predictive value     | 82.8%    |
| Negative predictive value     | 84.3%    |
| Likelihood ratio (+)          | 7.2      |
| Likelihood ratio (-)          | 0.279    |
| Accuracy                      | 83.8%    |

**Table 4: Correlation between intraoperative cytology and histopathology of margins**

| Intraoperative crush cytology | Histopathology | Total |
|-------------------------------|----------------|-------|
| Margins                       | Positive | Negative |     |
| Positive                      | 24      | 8        | 32   |
| Negative                      | 15      | 57       | 72   |
| Total                         | 39      | 65       | 104  |

**Table 5: Correlation between intraoperative cytology and histopathology of lymph nodes**

| Intraoperative imprint cytology | Histopathology | Total |
|---------------------------------|----------------|-------|
| Lymph nodes                     | Positive | Negative |     |
| Positive                        | 9       | 2        | 11   |
| Negative                        | 1       | 29       | 30   |
| Total                           | 10      | 31       | 41   |
for smears. The margins have lining squamous epithelium and the cells of well-differentiated squamous cell carcinoma may show minimal atypia. Figure 1a shows margins positive for malignancy. Two false-positive lymph nodes were seen in our study. On review of the false-positive lymph node, we found that the cells were very few on smear and was probably due to contamination. In these cases, as paraffin sections were negative, serial sections were done to rule out the presence of occult metastasis in the deeper sections. Increasing the number of sections of sentinel nodes examined may improve the chances of identifying small malignant deposits in histopathology. False-positive can be prevented by taking a disposable scalpel blade while bisecting for each lymph node. The main reason for the false-negative results of the imprint of the lymph node was the low cellularity of the imprint samples or because of sampling error. Terada et al.\(^\text{[12]}\) evaluated specificity, NPV, and diagnostic accuracy of 99%, 92%, and 92.6%, respectively by imprint cytology of sentinel lymph node of oral cancer patients. This compares with our study. Figure 1b shows metastatic deposits of squamous cell carcinoma in the lymph node.

We studied 4 cases of thyroid in which FNAC was suspicious or inadequate with clinical suspicion for malignancy. A definitive diagnosis was not possible due to the low cellularity of abnormal cells on repeated guided aspirates. All four cases represent different problems in diagnosis. In spindle cell neoplasm [Figure 1c] with minimal pleomorphism and low cellularity, cytologically differential diagnosis ranges from benign to anaplastic. IO cytology helped in definitive diagnosis and management decisions. When the material is inadequate and malignancy is clinically suspected, IO crush smear provides adequate representative material as in our cases. In Hashimoto’s thyroiditis, sampling from intervening areas yielded thyroiditis element while selection based on gross from nodular areas showed a large cell with cytological features of malignancy [Figure 1d].

We received four cases of breast lump which were suspicious on aspiration cytology and core biopsy showed inadequate material or was inconclusive. These cases were diagnosed as duct carcinoma intraoperatively and confirmed on histopathology.

In two cases of salivary neoplasms, a definite diagnosis could be given on intra-operative smears. In the first case, malignancy was suspected radiologically which was diagnosed as pleomorphic adenoma intraoperatively. In the second case, patient was very young and had a very large parotid mass [Figure 2a].

Another case where FNAC was repeatedly showing necrosis, intraoperative diagnosis of poorly differentiated carcinoma, confirmed as gall bladder carcinoma on histopathology was given. A retroperitoneal large spindle cell mass on FNAC was typed as schwannoma intraoperatively. Clinically the mass close to the spine was suspected to be tuberculous in nature. Another schwannoma [Figure 2b], adrenal cortical carcinoma [Figure 2c] and gastric adenocarcinoma [Figure 2d] were accurately diagnosed.

**Table 6: Comparison of cytohistopathology diagnosis in surgical specimen**

| Case no. | Organ            | FNAC diagnosis              | Intraoperative Diagnosis | Histopathology          |
|---------|------------------|------------------------------|--------------------------|-------------------------|
| 1       | Thyroid          | Spindle cell neoplasm        | Medullary carcinoma      | Medullary carcinoma     |
| 2       | Thyroid          | Hemorrhage and necrosis      | Anaplastic carcinoma     | Anaplastic carcinoma    |
| 3       | Thyroid          | Hemorrhage                   | Anaplastic carcinoma     | Anaplastic carcinoma    |
| 4       | Thyroid          | Hashimoto’s thyroiditis      | Lymphoreticular malignancy | Lymphoreticular malignancy |
| 5       | Breast           | Proliferative breast disease| Invasive duct carcinoma  | Invasive duct carcinoma |
| 6       | Breast           | Proliferative breast disease| Invasive duct carcinoma  | Invasive duct carcinoma |
| 7       | Breast           | Proliferative breast disease| Invasive duct carcinoma  | Invasive duct carcinoma |
| 8       | Breast           | Lobular carcinoma            | Lobular carcinoma        | Lobular carcinoma       |
| 9       | Salivary gland   | Salivary neoplasm            | Pleomorphic adenoma       | Pleomorphic adenoma     |
| 10      | Salivary gland   | Salivary neoplasm            | Adenoid cystic carcinoma | Adenoid cystic carcinoma |
| 11      | Abdominal lump   | Hemorrhage                    | Poorly differentiated malignancy | Papillary adenocarcinoma |
| 12      | Retroperitoneal mass | Hemorrhage                  | Spindle cell neoplasm    | Schwannoma              |
| 13      | Gastric biopsy   | Not done                     | Adenocarcinoma           | Adenocarcinoma          |
| 14      | Adrenal lump     | Hemorrhage                    | Adrenocortical carcinoma | Adrenocortical carcinoma |
| 15      | Frontal sinus mass | Not done                  | Schwannoma               | Schwannoma              |
In our study accuracy of the imprint was 83.8% which is close to the study of Chandrakar et al. (84%).\textsuperscript{13} Liu et al. (88.5%)\textsuperscript{14} and Badami et al. (86.31%).\textsuperscript{15} The specificity of our study 89.6% is close to the study by Adhya et al. (97.4%).\textsuperscript{16} Sharma et al. (83.3%)\textsuperscript{17} and Geothe (94%).\textsuperscript{18} Low sensitivity (75%) in our study may be due to less sample size, more specimens of resection margin which gave high false negative.

Limitations of intraoperative cytology are the same as those of cytology, that is, sampling error, inability to distinguish \textit{in situ} from invasive lesions and inability to provide details of depth of invasion. Micro-metastases are unlikely to be detected by this method. The Pitfalls in the study were in the initial phase when contaminations resulted in a few false positives. However, with close coordination between surgeon and cytologist, these errors were minimized. The sample size of our study was limited and large studies are needed especially in margin evaluation.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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