

Training in Preparing Crush Smears for Cytology as a Rapid Diagnostic Method of Malignancies: Can it be Effective for Guiding Therapeutic Decisions?

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ABSTRACT

Background: Crush or squash cytology smears are routinely performed for tissues of the central nervous system and gastrointestinal tract lesions. Our clinicians are also sending similar smears from tissues of aerodigestive tract malignancies for rapid diagnosis and to guide therapeutic decisions. The study aims to evaluate the efficacy of crush cytology in rapid preoperative diagnosis of non-CNS neoplasms and to analyze factors contributing to cyto-histological discordance before and after training.

Methods: A prospective diagnostic test study was conducted in 110 patients with suspected malignancy, mostly of the aerodigestive tract. Crush smears for cytology were prepared by surgeons from biopsy specimens, and the residual tissue was fixed in formalin for histopathology. The initial 30 cases in the pilot study were analysed for the causes of cyto-histo discordance. Training was imparted to surgeons regarding the preparation and fixation of cytology smears. Agreement analysis was performed for the diagnostic performance of cytology smears and tissue sections.

Results: Out of the 110 cases studied, 80 were from the aerodigestive tract. Overall, the sensitivity and specificity of crush cytology were 91.01% and 81.81%, respectively. The overall agreement between crush cytology and histopathology was almost perfect, with a Cohen-Kappa value of 0.97.

Conclusion: Crush cytology smears from solid tissue biopsies can provide rapid, accurate diagnoses for planning the preoperative workup of patients with malignancies. However, training of surgeons in smear preparation is a prerequisite to optimizing the use of hospital resources.

Key-words: Crush cytology, Squash cytology, Neoplasm, Histopathology, Malignancies

INTRODUCTION

Considering the rising trend in the rate of malignancies and to avoid their life-threatening consequences, early diagnosis and prompt intervention are a must.

It is well recognized that the examination of different cytological specimens provides sufficient information to drive treatment decisions.^[1,2] Although histopathology is the gold standard for tissue diagnosis, its turnaround time is a limiting factor.^[3,4] Intraoperative histopathology diagnosis using a frozen section is not available in many under-resourced hospitals and institutes and requires technical expertise. Cytology, as a rapid diagnostic test, has gained tremendous momentum in recent times due to its speed, accuracy, and cost-effectiveness.^[5,6] Crush

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smears prepared from biopsy material are one such established method for rapid diagnosis.

Crush cytology has been extensively studied for intra-operative diagnosis of Central Nervous System (CNS) lesions and is now a well-established technique with a high diagnostic accuracy of 90%.^[7] However, a review of the literature shows that crush cytology has not been utilized for malignancies other than the intraoperative diagnosis of CNS lesions and gastrointestinal tract malignancies.^[8-12] The primary aim of this study was to determine the efficacy of crush cytology in the rapid pre-operative tissue diagnosis of neoplasms other than CNS. The secondary aim was to conduct an in-depth evaluation of the technical and other causes of cytological-histological discord in cases before and after training.

MATERIALS AND METHODS

Study Design and Setting- This was a prospective cross-sectional study conducted on 110 patients with suspected malignancies who came to our hospital between January 2020 and August 2021. The included patients had malignancies of the aerodigestive tract, breast, metastatic lymph nodal masses, and salivary gland swellings.

Methodology- Before starting this study, clinicians used to prepare crush smears from biopsy material placed on a glass slide, crushed, and spread onto another slide. 2-3 such smears were prepared and sent to the cytology laboratory in 95% ethyl alcohol. After interpreting 30 such cases, we observed that smear quality was poor; they contained thick tissue chunks and drying artifacts of most cells, making it difficult for us to reach a diagnostic conclusion. It was decided to hold training sessions for ENT surgeons in the operating theatre. We demonstrated to them the correct technique for handling excised tissue and preparing crush cytology smears. Emphasis was placed on preparing thin smears without excessive crushing of tissue particles. The importance and demonstration of rapid fixation of slides in absolute alcohol while they were still wet were also shown. They were also asked to avoid delays in immersing the residual biopsy tissue in formalin. When these instructions were followed, there was a significant difference in the quality of crush cytology smears and subsequent microscopic diagnosis.

In the cytology laboratory, the crush smears were stained with hematoxylin and eosin and Papanicolaou stains. All cytological interpretations were double-checked by a senior and a junior cytologist. Smears were reported as positive, suspicious, negative for malignancy, and unsatisfactory for evaluation. Whenever possible, specific typing of malignancy was done. Reports were dispatched within 5-6 hours of receiving the smears.

Statistical Analysis- The cytological diagnosis was correlated with the histopathological diagnosis. Kappa statistics were used to test the agreement between crush cytology and histopathology. The test parameters of crush cytology diagnosis (sensitivity, specificity, positive predictive value, and negative predictive value) were calculated. We conducted an in-depth evaluation of the technical and other causes of cyto-histo discord in cases before and after training.

Ethical Considerations- Approval for the study was obtained from the Institutional Ethics Committee (IEC).

RESULTS

This study comprised 30 cases before training and 110 cases after training. Most cases were from the pharynx, with 36 cases, followed by 26 from the oral cavity (Table 1).

Table 1: Site-wise distribution of lesions

Site	Number	
	Before training	After training
GIT	5	8
Nose & PNS	7	18
Pharynx	6	36
Oral Cavity	7	26
Tongue	0	05
Breast	1	03
Lymph node	3	07
Salivary gland	0	01
Others	1	06
Total	30	110

The cytology smears were classified as unsatisfactory, negative for malignancy, suspicious for malignancy, or positive for malignancy. In 88 cases, the diagnosis was positive for malignancy (Table 2).

Table 2: Broad diagnosis of crush cytology smears

Category	No.of Cases
Unsatisfactory	08
Negative for malignancy	09
Suspicious for malignancy	05
Positive for malignancy	88
Total	110

Of these 88 cases, specific typing of malignancy was possible in 45 cases (Table 3).

Table 3: Specific morphologic diagnosis rendered by crush cytology smears

Crush smear Diagnosis	No
Squamous Cell carcinoma	37
Adenocarcinoma	07
Lymphoreticular malignancy	1
Total	45

Detailed histopathologic diagnosis and typing were possible in 110 cases. In 20 cases, a diagnosis of malignancy could be given, while further typing was not possible (Table 4).

Table 4: Histopathologic diagnosis of biopsies used for preparing crush cytology smears.

Histopathology Diagnosis	Number of Cases
Unsatisfactory (tissue insufficient for diagnosis)	02
Negative for malignancy	09
Positive for malignancy	11
Squamous cell carcinoma	60
Adenocarcinoma	15
Mucoepidermoid carcinoma	05
Spindle cell malignancies	04
Rhabdomyosarcoma	02
Non-Hodgkins Lymphoma	01
Hepatocellular carcinoma	01
Total	110

In 2 cases, the biopsy was unsatisfactory; therefore, an opinion was not possible. 81 cases were positive on both crush cytology and histopathology. 9 cases were negative by both tests, probably due to inadequate biopsy material. 02 cases were negative by histopathology but positive by crush cytology. 8 cases

were negative by crush but positive by histopathology (Table 5).

Table 5: Correlation of diagnosis made by crush smears with that of histopathology

Crush cytology	Histopathology	
	Positive	Negative
Positive	81	02
Negative	08	09
Total	89	11

Before surgeons' training, the analysis of 149 crush cytology cases had a sensitivity of 69.14% & specificity of 78.95%, with a higher PPV of 93.33% and a diagnostic accuracy of 71%, with a significant p-value but a slightly lower kappa value. However, agreement was 76% (Table 6).

Table 6: Statistical analysis before adequate training of surgeons

Sensitivity	69.14%
Specificity	78.95%
Positive predictive value	93.33%
Negative predictive value	37.50%
Diagnostic Accuracy	71%
P value	<0.001 (Significant)
Kappa value	0.47
Agreement	76%

The statistical analysis of 110 cases, after excluding 29 cytology-histology discordant cases obtained before training the surgeons, showed that sensitivity increased to 98.24%, specificity to 100%, and PPV to 100%, with diagnostic accuracy reaching 98.6% and a significant p-value, with a Kappa value indicating almost perfect agreement between the tests (Table 7).

Table 7: Statistical analysis after adequate training of surgeons

Sensitivity	98.24%
Specificity	100%
Positive predictive value	100%
Negative predictive value	93.75%
Diagnostic accuracy	98.61%
p-value	<0.0001 (Significant)
Kappa value	0.95 (almost perfect)
Agreement	98.61%

DISCUSSION

Currently, cytology is the first-line diagnostic tool and is considered a standard procedure for the rapid diagnosis of a variety of cytological specimens obtained from easily accessible malignancies.^[13] Rapid stains can be used for the rapid cytologic diagnosis of FNAC smears, scrape material, imprints, intraoperative cytology, and crush preparations of biopsies. However, each of these techniques has its limitations. Fine-needle aspiration cytology (FNAC) with rapid stains can be used only for superficial, palpable lesions.^[14] Scrape smears are good for ulcero-proliferative lesions and provide diagnostic material only if viable cells can be scraped from the edges.^[6] Imprint cytology has the disadvantage of releasing cells from a single plane.

Crush smears can be prepared from tiny tissue pieces removed from any and every site, superficial or deeply seated, or accessible by endoscopes, and are generally representative of the lesion.^[15] The crush smear technique, though simple, requires training for clinicians who intend to use it for their biopsies. A thin smear of chunky tissue material, along with rapid wet fixation of the smear, is the bottom line behind the success of this simple, cost-effective tool.

Our ENT surgeons had started sending crush smears from biopsies from the oral cavity, larynx, oropharynx, etc. More often than not, smear quality was suboptimal. In the pilot study of 30 cases, cytology reports were deemed unsatisfactory for evaluation in 26 cases, and in the remaining 4 cases, the material was inadequate for histopathology. An analysis of the initial 30 cases showed that in 26 cases, a diagnosis was not possible using the clinicians' crush smears and was made on histopathology alone. In 16 out of 26 cases, there was an error in the technique of smear preparation, such as crushing and drying artifacts and forming thick tissue chunks on the smear. 10 out of 26 cases had no representative material in the cytology smears.

As we trained surgeons, the quality of smears improved drastically, and there were 10 cases in which a diagnosis was not possible. Only 2 cases had poor-quality crush cytology smears, and in 6 cases, cytology material was inadequate. In the remaining 2 cases, crush smear preparation exhausted the material. Of the 10 cases with a disagreement in diagnosis between cytology and histopathology, 8 were mainly due to differences in the representation of material in either technique.

A review of the literature revealed very few studies in which crush cytology smears were used for the rapid preoperative diagnosis of biopsy material collected from tissues other than CNS lesions. In our study, 8 endoscopic biopsy specimens were used for rapid diagnosis via crush smears. *Batra et al.*^[12] studied the efficacy of crush cytology for the evaluation of gastrointestinal lesions and reported a sensitivity of 89.7% for crush smears in diagnosing gastroesophageal lesions. They observed that crush cytology and histopathology have equivalent diagnostic reliability for gastrointestinal tract malignancies.

Desai et al.^[11] used crush cytology to diagnose gastrointestinal malignancies in endoscopically suspected malignant lesions and, when compared with histopathology, achieved a diagnostic accuracy of 96.9%. The sensitivity, specificity, positive predictive value, and negative predictive value of crush smear results were 97.3%, 90%, 99.2%, and 72.5%, respectively. *Chaithra et al.*^[6] studied the efficacy of colonoscopic crush cytology as a convenient and near-accurate method to evaluate colonic neoplasms. The sensitivity and specificity of crush cytology for colorectal malignancies were 96% and 63.2%, respectively. A PPV of 91.14% and NPV of 80% were seen in their study. Specificity was low because adenomas, which are non-malignant but dysplastic, were considered malignant on cytology. *Bhat et al.*^[16] compared crush cytology with histopathology for endobronchial growths. They found that, of 49 cases, 40 histopathologically proven malignant cases were also positive for malignancy on crush smear cytology. They reported that the sensitivity, specificity, positive predictive value, and negative predictive value of crush smear results were 81.6%, 100%, 100%, and 77.5%, respectively.

In the present study, the majority of crush cytology specimens (94 cases) were from the aerodigestive tract. All our cases were clinically and radiologically diagnosed malignancies requiring rapid tissue diagnosis with crush smears to expedite the next step in treatment, while the histopathology report is still pending. In comparison with the above studies, our study showed a sensitivity of 98.24%, specificity of 100%, and PPV of 100%, with a diagnostic accuracy of 98.61%, and a significant P value and Kappa value indicating almost perfect agreement between the tests.

Table 8: Comparison with other similar studies

Statistical Parameter	Chaithra et al. [6]	Bhat et al. ^[16]	Desai et al. ^[11]	Present Study (2021)
Sensitivity	96%	81.6%	97.3%	98.24%
Specificity	63.2%	100%	90%	100%
PPV	91.1%	100%	99.2%	100%
NPV	80%	77.5%	72.5%	93.75%
Diagnostic accuracy	--	--	96.9%	98.61%

When the crush cytology test is negative, it is largely due to split-sample assessment (done for cytology and HP), which has its limitations. There is a risk of inadequate or non-representative tissue for cytology or histopathology. In our study, 2 cases were positive for crush cytology but negative for histopathology. On the other hand, in 8 cases cytology did not yield representative cells, but histopathology provided diagnostic material. Therefore, with clinically and radiologically proven malignancies, it is not a matter of false-positive or false-negative diagnosis; rather, it is a limitation of the split-sample technique. Despite these limitations, crush cytology not only provides a rapid diagnosis but also serves as a measure of the adequacy of the clinician's biopsy.

CONCLUSIONS

Thus, crush cytology may increase costs beyond histopathology. Still, because it does not require additional equipment and is inherently simple and rapid, it can be cost-effective for the hospital and the patient's further workup. Crush cytology is a highly sensitive, specific, rapid, and cost-effective procedure for diagnosing malignancies in suspected malignant lesions, with high diagnostic accuracy. With proper training in crush smear preparation and rapid diagnosis, the long wait for biopsy reports can be shortened. The results of our study will likely necessitate the widespread implementation of this diagnostic technique in the near future.

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