Crush cytology: an expeditious diagnostic tool for gastrointestinal tract malignancy

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ABSTRACT

Background and study aims Crush cytology is a simple and rapid method used for diagnosis of central nervous system lesions. We have evaluated the diagnostic accuracy of crush cytology for gastrointestinal tract lesions.

Patients and methods This was a prospective, cross-sectional, single center study, conducted on the patients who had suspected malignant lesions between August 2018 and March 2020. The crush cytologic diagnoses were correlated with histology to determine the diagnostic accuracy.

Results During the period of interest, a total of 451 patients (26.4% esophagus & GE junction, 16.6% stomach, 5.9% ampulla & duodenum, and 50.9% colorectal) had a suspected malignant lesion on endoscopic examination. Histology confirmed 92.9% cases as malignant lesions and 7.1% as nonmalignant. On crush cytology, 84.5% were positive for malignancy, 8.9% were negative for malignancy and 6.6% were reported as suspicious for malignancy. The overall sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of crush cytology were 97.3%, 90%, 99.2%, 72.5% and 96.9%, respectively.

Conclusions Crush cytology is a highly sensitive, specific, rapid and cost effective technique to diagnose gastrointestinal malignancies in endoscopically suspected malignant lesions. However, it cannot entirely substitute histopathological examination for definite tumor typing, grading, confirming invasion and in cases in which cytology is suspicious. Crush cytology is an added asset to the histology to maximize diagnostic accuracy and accelerating decision making for the management of lesions.

Introduction

As a group, gastrointestinal cancers are not only one of the most common cancers but also one of the most common causes of cancer related mortality. According to the GLOBOCAN 2018 data, colorectal malignancy is the third and gastric cancer is the fifth most common cancer worldwide, and colorectal cancer is the second and gastric cancer the third leading cause of cancer related mortality. The global cancer burden is estimated to have risen to 18.1 million new cases and 9.6 million deaths in 2018, according to GLOBOCAN 2018 data. In India the estimated incidence of cancer is 89.4 per 100,000 and cancer-related mortality is 61.4 per 100,000 population [1]. In India, the incidence of gastric and colorectal cancer ranks sixth and seventh among most common cancers. Worldwide, the most commonly diagnosed gastrointestinal cancers include colorectal, gastric, liver (e. g. hepatocellular carcinoma), esophageal, and pancreatic. The advent of flexible fibreoptic video

endoscopy with narrow band imaging (NBI) and endoscopic ultrasonography has greatly enhanced the ability to closely visualize several part of the gastrointestinal tract and obtain specimens under direct vision for histologic evaluation[2]. It is well accepted that the ultimate diagnosis of malignancy is based on histopathologic evaluation (HPE). The utility of cytology in the diagnosis of luminal gastrointestinal tract tumors is not addressed adequately in comparison to the HPE. Cytological evaluation can be done using brush cytology or crush smear technique. The pooled sensitivity and specificity of brush cytology for diagnosis of gastrointestinal malignancy has been reported as 83.4 % and 80.9 %, respectively. The efficacy of all the cytological techniques is still continuously being assessed [3, 4].

Crush cytology is utilized as an excellent adjunct tool for central nervous system lesions, especially for intraoperative diagnosis [5]. Crush cytology is simple, easy to perform, cost effective and gives rapid results with minimal resources which could be an added asset to the histopathology [5]. Its efficacy and utility in guiding extent of neurosurgical resection has been proven, but there are a few studies in the literature on application of crush cytology for gastrointestinal malignancy [3, 6]. Thus, the present study was undertaken to look at the reliability of crush cytology against the gold standard histopathology of gastrointestinal lesions.

Patients and methods

Patients

This was a prospective, cross-sectional, single-center study. Patients were enrolled between August 2018 and March 2020. A total of 11,690 endoscopic procedures were carried during this period and all patients who underwent either gastroscopy, colonoscopy, or side viewing scopy and were suspected to have malignant lesions on endoscopy were included in the study. Endoscopy was performed using a standard Gastroscope (Olympus EVIS EXERAIII HQ190), Colonoscope (Olympus EVIS EXERAIII CF-H190 L) or Side viewing scope (Olympus EVIS EXERAIII TGF-Q180) depending on the site of the lesion. On endoscopy, the patients with visible mucosal lesions such as ulcerative growth, stricture, or polypoidal mass lesions were assessed and biopsy specimen was taken from suspicious lesions using a biopsy forcep (with central spike). First, biopsy tissue was used for preparation of crush smears and smears were fixed in methanol and rest of the four biopsy samples were collected for HPE in 10 % buffered formalin. Then, the samples were sent to the laboratory, where the crush smears were processed for cytology immediately and biopsy tissue was processed and sections cut from paraffin embedded blocks and stained with hematoxylin and eosin (H&E) for evaluation. Biopsy slides were available for evaluation usually after 24 hours. The final HPE report was available within 4 days.

Preparation of crush smears

After taking biopsy, the endoscopist prepared the slide and sent it to the laboratory for cytofixation. For crush smears, a single biopsy tissue was gently crushed (squashed) between the two glass slides. A slide used for crushing tissue was kept

at right angle to the slide on which tissue was placed. This slide was immediately fixed in methanol. It was then transported to the laboratory and then stained with H&E. Staining time for crush cytology smear was about 45 minutes. The reporting time for the crush cytology smear was about one hour from the time of taking endoscopic biopsy. The crush smears and histopathological sections were examined by single well-versed pathologist who was blinded for the name, demographics and crush cytology findings but had an access to the endoscopic findings. All the findings were checked twice and final consensus diagnosis report was evaluated. The crush cytology diagnosis was recorded under three categories: positive for malignancy, suspicious of malignancy and negative for malignancy. A positive diagnosis on crush was given when there were unequivocal malignant cell clusters with good cellularity on smears. Smears which revealed low cellularity, or which showed only few atypical clusters, which were quantitatively or qualitatively insufficient to make a confident diagnosis of malignancy were reported as suspicious of malignancy. Negative for malignancy was reported in cases with definite absence of malignant or atypical cells or features consistent with inflammatory lesion. The results of crush cytology were compared with the histopathology results and histopathology was considered as the gold standard. For statistical purposes, suspicious category was considered as positive for malignancy.

Statistical analysis

Sensitivity, specificity, accuracy, positive predictive value and negative predictive value were calculated by comparison of HPE reports. Comparison and significance between crush cytology and histopathologic diagnosis, using chi-square test, were calculated with the SPSS statistical package, version 16.0 (SPSS Inc., Chicago, Illinois, United States).

Results

During the period of interest, a total of 451 patients were included with suspicious malignant lesions on endoscopic examination. The lesions were detected in the esophagus and gastroesophageal junction (26.4 %), stomach (16.6 %), ampulla and duodenum (5.9 %), and colon and rectum (50.9 %). There were 64 % men with a mean age of 59.2 ± 15.7 years. Of 451 lesions, histology confirmed 419 cases of malignant lesions and 32 cases of nonmalignant lesions (▶ Table 1). The nonmalignant lesions included three tuberculosis, two amoebic colitis/ameboma, 12 inflammatory lesion/ulcers, six adenomatous lesions with low-grade dysplasia, four high-grade dysplasia, one infarcted necrotic lipoma, one hyperplastic polyp, one inflammatory cap polyposis, and two serrated adenomas.

On crush cytology, 381 were diagnosed as positive for malignancy, 30 were suspicious for malignancy, and 40 were negative for malignancy. The cytomorphologic diagnosis is illustrated in ▶ Table 2. The correlation of crush cytology and histopathological diagnosis of malignant lesions for the gastrointestinal tract are shown in ▶ Table 3 ($P = 0.860$; χ2 test). The sensitivity and specificity of crush cytology for gastrointestinal tract malignancies were 97.3 % and 90 %. The observed positive predictive value was 99.2 % and negative predictive value was

▶ Table 1 Crush cytological diagnoses of the study population.

Biopsy site	Diagnostic Category			
	Positive for malignancy	Suspicious for malignancy	Negative for malignancy	Total
Esophagus + GEJ	113	3	3	119
Gastric	64	6	5	75
Colorectal	183	18	29	230
Ampullary + duodenal	21	3	3	27
Total	381	30	40	451

GEJ, gastroesophageal junction.

▶ Table 2 Cytohistologic diagnosis of study population (crush cytology and histopathology).

Biopsy site	Malignancy on crush and histology (n)	Malignancy on crush and nonmalignant on histology (n)	Nonmalignant on crush and malignant on histology (n)	Nonmalignant on crush and histology (n)	Total (n)
Esophagus + GEJ	116	0	0	3	119
Gastric	70	0	0	5	75
Colorectal	200	1	10	19	230
Ampullary + duodenum	22	2	1	2	27
Total (n)	408	3	11	29	451

GEJ, gastroesophageal junction.

**▶ Table 3** Cytomorphologic categorization of gastrointeestinal malignancy using crush cytology.

Cytomorphology	Site				Total
	Esophagus + GEJ (n)	Gastric (n)	Colorectal (n)	Ampullary + duodenum (n)	
Squamous cell carcinoma	85	0	2	0	87
Adenocarcinoma	17	50	172	18	257
Poorly differentiated carcinoma	8	9	6	4	27
Non-Hodgkin lymphoma	2	2	1	0	5
Neuroendocrine tumor	0	1	1	0	2
Spindle cell neoplasm/GIST	0	2	1	0	3
Total	113	64	183	22	381

GEJ, gastroesophageal junction; GIST, gastrointestinal stromal tumor.

72.5 %. In addition, the crush cytology achieved 100 % accuracy for the esophagus, gastroesophageal junction and gastric lesions.

In esophagus, well to moderately differentiated squamous cell carcinomas could be diagnosed as squamous type (▶ Fig.1a) with confidence but in moderately poor to poorly differentiated cases were difficult to diagnose the exact type on crush cytology alone. Crush cytology smears from small ampullary and periampullary lesions were most difficult to diagnose, particularly small polyps, small proliferative lesions, because these lesions had low cellularity, well differentiated na-ture, and lack of architectural details. Such a lesion showed presence of few atypical clusters which could represent regenerative atypia. The lesions with grossly malignant appearing large masses on endoscopy did not impose much diagnostic problem. Moreover, the adenocarcinomas (▶ Fig.1b) could be diagnosed successfully on cytology alone and majority of cases were observed in colorectal lesions. But for poorly differentiated carcinoma, non-Hodgkin lymphoma (NHL) (▶ Fig.1c), neuroendocrine tumor (NET) (▶ Fig.1d), spindle cell neoplasms (▶ Fig.1e) biopsy and immunohistochemistry confirmation was required. Cases of lymphoma could be diagnosed as malignant
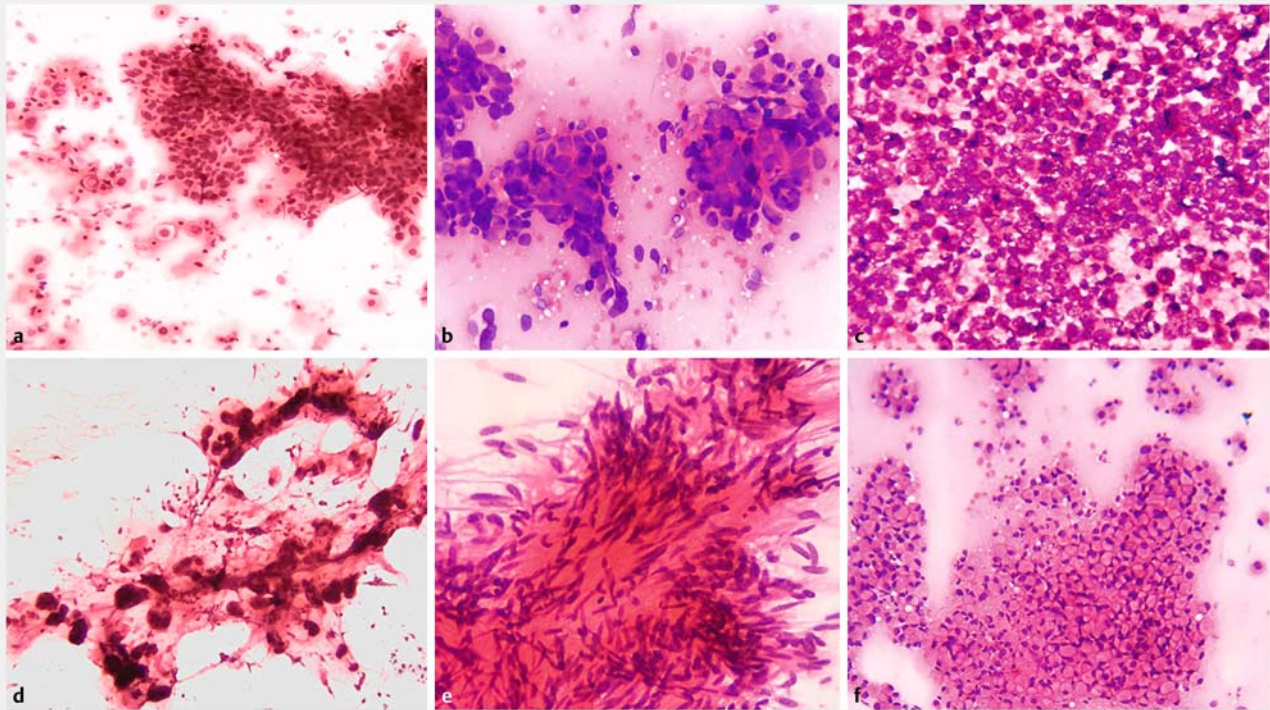
►**Fig. 1** Crush cytology. **a** Squamous cell carcinoma. **b** Adenocarcinoma. **c** Non-Hodgkin lymphoma. **d** Mucin-secreting adenocarcinoma.
e Spindle cell neoplasm. **f** Signet ring carcinoma.

round cell tumors which revealed a possibility of NHL that could be confirmed by HPE and immunohistochemistry. It was observed that cases with either in situ/intramucosal lesion revealed only focal high-grade dysplasia (►**Fig.1f**) on crush due to low cellularity. Whereas, in invasive malignancies the smears were highly cellular with majority of cell clusters showing unequivocal features of malignancy like nuclear enlargement, pleomorphism, hyperchromasia, marked anisonucleosis, crowding, overlapping, loss of polarity, abnormal mitoses.

Discussion

Determining the true nature of gastrointesttinal tract lesions is crucial. Early diagnosis of gastrointestinal tract lesions with endoscopic evaluation plays a decisive role for their management. Depending upon the location and type of lesions various cytological techniques may be employed for primary diagnosis of lesions. Numerous studies have highlighted the diagnostic pitfalls of brush and touch smear cytology [3, 4, 7]. However, there are a few reported studies in the literature on the role of crush cytology in diagnosis of gastrointestinal tract malignancy. Crush cytology is a simple technique and can be performed with the available equipment in endoscopy and pathology laboratory [6]. In the present study, we have evaluated the diagnostic efficacy of crush cytology for malignancy of the gastrointestinal tract. We assessed sensitivity of 97.3%, specificity of 90% and overall diagnostic accuracy of 96.9%.

Batra et al. [3] and Chaithra et al. [6] proposed that crush cytology and histopathology have equivalent diagnostic reliability for malignancy of large intestine, stomach and esophagus. In the present study, we have evaluated the efficacy of crush smears for whole gastrointestinal tract. Batra et al. [3] reported sensitivity of crush smears 89.7% for gastroesophageal lesions. In the same way, Chaithra et al. [6] compared crush cytology with histopathology of resected specimens of large intestine and reported the sensitivity of 96% and specificity of 63.2%. In our study the sensitivity and specificity of crush cytology for esophageal, GE junction and gastric lesions was 100%, while the sensitivity and specificity was 95.2% and 95% respectively for colorectal lesions (►**Table 4**).

Cytology can be conducted by many ways; brush, crush and touch imprints are just a few names. Brush cytology is indeed an advantageous procedure as it provides wide area to investigate. Usually, it takes longer time, which is uncomfortable for the patients. Likewise, touch imprints are unpredictable, in cases of tumor with deeper infiltration. In consequence of these major pitfalls we might land up with inadequate diagnosis. Apart from the consequences of cytological procedures, it requires additional efforts and equipment's to yield the diagnosis. Whereas crush cytology requires minute amount of tissue, no additional equipment and efforts are needed and these together make crush cytology cost-effective. Most importantly, crush cytology produces rapid diagnosis within an hour in contrast to histopathological examination, which takes 3 days. However, histopathological examination cannot be replaced

▶Table 4 Diagnostic correlation between crush cytology and histopathology for gastrointestinal malignancy.

Site	Sensitivity (%)	Specificity	PPV	NPV
Gastrointestinal tract	97.3	90.0	99.2	72.5
Esophageal + GEJ	100	100	100	100
Gastric	100	100	100	100
Colorectal	95.2	95.0	99.5	65.5
Ampullary + Duodenal	95.6	50.0	91.6	66.6

GEJ, gastroesophageal junction; PPV, positive predictive value; NPV, negative predictive value

completely by crush cytology for tumor typing, grading, and confirmation of invasion.

It would be expected to have a suspicious finding on crush cytology. Even with histologic examination, a few cases are inconclusive because of suspicious findings. The inference is that the smear is low cellularity or there was a sampling error. In the present study, 30 cases of crush cytology with suspicion of malignancy but not definitive were identified and considered positive for statistical analysis only, not for surgical decision making. In cases with suspicious but not conclusive findings, the clinician has always been advised to wait for the histopathology report. Statistics applied without including these cases had sensitivity, specificity, positive predictive value, and negative predictive values of 97.1%, 96.7%, 99.7%, and 72.5% respectively.

Crush artifacts in squash cytology were not a major problem. In fact, cell morphology was well preserved in almost all cases and acceptable for interpretation, except in an occasional case of necrotic mass lesion. Crush cytology smears from very-well-differentiated tumors showed bland nuclear features on cytology. But those with low cellularity from markedly ulcerated and necrotic lesions or from stricturing lesions showed few atypical clusters that could not be confidently diagnosed as malignant on crush alone and were the reason of false negatives and an occasional false-positive result. Thus, well-differentiated tumors or tumors with low-grade morphology pose a major problem in the diagnosis using crush cytology, thus resulting in a low negative predictive value. In the present study, 11 cases of malignancy were negative on crush but positive on histology. Among them, 10 cases were colorectal malignancies. The possible explanation would be as follows: Six cases were well-differentiated adenocarcinoma with low-grade morphology, which was difficult to diagnose on crush cytology. Three were low-grade lymphoma, which were difficult to distinguish from inflammatory lesions without referring architectural features. One case was a NET, which was predominantly submucosal and had lymphovascular tumor emboli, so it was not seen in the crush smears. In all such cases, "wait for biopsy policy" should be followed. Well-differentiated adenocarcinoma could be diagnosed on histology, wherein architecture and invasion can be assessed. It is emphasized that in cases of negative or suspicious results on cytology, the biopsy report should always be awaited before decisions are made about radical surgical management, unless there is a surgical emergency. We suggest that biopsy should always be performed in formalin for histopathological examination along with crush to avoid unnecessary repeat endoscopy if crush cytology is negative or suspicious. Moreover, if crush cytology is positive, further management can be immediately planned and we may not have to wait for a biopsy report. Lymphoma, gastrointestinal stromal tumor, and NET showed features that suggest the diagnosis in some cases but definitely require confirmation of biopsy and immunohistochemistry. In these cases, we can only suggest a diagnosis, which needs to be confirmed on histology. In the present study, crush cytology diagnosed NHL, three gastrointestinal stromal tumors, and two NETs. Low-grade lymphoma with bland cytology was difficult to diagnose on cytology and resulted in three false negatives. One case of NET was also false negative. On the other hand, poorly differentiated NETs cannot be accurately differentiated by cytology and are difficult to differentiate from adenocarcinoma, even on histopathologic examination.

Immunohistochemistry, therefore, is needed in these cases. In addition, NBI is another diagnostic modality to distinguish malignant lesions on endoscopic examination. However, NBI provided subjective findings which may have inter-observer variability. In contrast to that crush cytology provided objective findings to diagnose malignancy. Hence, further study is required to correlate findings of crush cytology with NBI.

Conclusions

In conclusion, crush cytology appears to be a highly sensitive, specific, rapid and cost effective procedure to diagnose gastrointestinal malignancies in endoscopically suspected malignant lesions. Moreover, crush cytology for lesions in the esophagus, gastroesophageal junction, and stomach is most reliable. Cytomorphologic evaluation on crush cytology along with good correlation of clinical and endoscopic findings is quick enough to deliver the diagnosis on the same day of endoscopy, which saves time and accelerates the decision making for the management of malignant lesion.

Competing interests

The authors declare that they have no conflict of interest.

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