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Diagnostic Accuracy of Crush Smear Cytology in Gastrointestinal Malignancy

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ABSTRACT

Crush smear cytology, is a useful adjunct to conventional histopathology for diagnosis of Gastrointestinal (GI) malignancies as it enhances cell yield and expedites diagnostic work up. Aim of our study is to determine the diagnostic accuracy of crush smear cytology by comparing with histopathology in GI malignancies. In this prospective, cross sectional analytical study, Crush smear slides and formalin preserved tissue samples of 180 cases obtained during GI endoscopic or colonoscopic procedures, were processed for cytology and Histopathology (HP) study. Relevant clinical history and endoscopic finding of each patient was obtained in standard format. The diagnosis on crush smear cytology was correlated with histopathological diagnosis. SPSS Statistics 24.0 has been used. Categorical variables studied by using frequency procedure, diagnostic efficacy of crush smear cytology have been done by using diagnostic test evaluation calculator - MEDCALC software. Out of 180 cases, 90 (50%) cases were reported as malignant on cytology whereas 95 (52.77%) cases on histopathology. 6 cases of malignancy were reported as negative (false negative) and 14 cases of non- malignant lesions were reported positive (false positive) on cytology smears. Basing on the above findings, Sensitivity, Specificity, PPV, NPV and diagnostic accuracy were calculated to be 93.68%, 83.53%, 86.41%, 92.21% and 94.82%, respectively. Crush smear cytology gives a high diagnostic yield in GI malignancy. Hence, cytology diagnosis saves time and gives proper timely feedback to the gastroenterologists for early management of the patients.

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Key Words
Gastrointestinal endoscopy, crush smears cytology, histopathology

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INTRODUCTION

The highest incidence of malignancy are seen at oesophageal, gastric and colorectal sites[1]. Gastric adenocarcinoma is the second most common cancer worldwide[2] and colorectal and gastric cancer are the leading causes of cancer mortality worldwide[3]. In India, gastric and colorectal cancer ranks sixth and seventh respectively[4]. Early diagnosis of malignancy from tissue samples increases the survival rate of patients. Various cytological techniques like brush, crush, touch and imprint cytology are used along with endoscopic biopsy, the gold standard but time consuming technique[5,6]. Crush (Squash) smear cytology used for Central Nervous System lesions, has evolved its application on diagnostic value on GI malignancies[4,7].

The present study was carried out to determine the accuracy of crush smear cytology in diagnosing malignancies of any site of Gastrointestinal Tract, by comparing with histopathology of those GI lesions and the reasons for discordance between cytological and histopathological diagnosis.

MATERIALS AND METHODS

After obtaining due consent from the participants and ethical clearance from IEC as per Declaration of Helsinki, this cross sectional analytical study was carried out in the department of pathology from 2019 to 2021 (two years) on 184 number of samples received from department of gastroenterology from clinically suspected GI malignancy cases in a tertiary health care centre of Eastern India. Tissue samples in cases with visible mucosal lesions such as ulcers and ulceroproliferative growths in the GI tract suspected of having malignant lesions of oesophagus, gastroesophageal junction, stomach, small intestine, colon and rectum (on routine upper endoscopy or colonoscopy) were included in the study. During endoscopy, for cytology few bits of tissue from suspected lesion were taken on a slide, smears were prepared by crushing the tissue in between two slides, out of which one slide was air dried for Diff quik staining and other slide was fixed in methanol for H and E or Papanicolaou stain and for histopathology the remaining tissues were fixed with 10% neutral buffered formalin for concurrent biopsy. Except 4 samples, all other tissues collected during endoscopy and colonoscopy procedure were considered as appropriate samples and subjected for comparative study on cytohistologic findings in Department of Pathology. Also, relevant clinical history of GI symptoms and endoscopic finding of each patient was also obtained in standard format prior to tissue evaluation.

The diagnosis of gastrointestinal lesions was done for each case separately by independent pathologist on the cytological and histopathological findings. Then, the diagnosis on crush smear cytology was compared with histopathological diagnosis along with review of patient's clinical, endoscopy or colonoscopy records. The malignant cytological features like cellularity, pattern, cell type and mucoid or necrotic background materials were interpreted with appropriate clinical inputs.

As described by Desai et al the crush cytology diagnosis was categorised under three headings[4]: (I) positive for malignancy, (Unequivocal malignant cell clusters with good cellularity on crush smears) (II) suspicious of malignancy (smears showing low cellularity, or with only few atypical clusters and which were quantitatively or qualitatively insufficient to make a confident diagnosis of malignancy) and (III) negative for malignancy (cases with definite absence of malignant or atypical cells or features consistent with inflammatory lesion). The cytomorphologic features for establishing malignancies of different GI sites are as mentioned below[1,8].

Squamous cell carcinoma of oesophagus: Discretely lying polyhedral to fibre cells, thick orangeophilic cytoplasm, necrotic background

Gastric adenocarcinoma: Intestinal type: loosely cohesive cells, columnar to cuboidal cells, high n/c ratio, irregular outline, hyperchromatic, prominent nucleoli.

Diffuse type: single round cells, abundant vacuolated cytoplasm, peripherally pushed crescent shaped hyperchromatic nucleus, mucinous background.

Adenocarcinoma of Small intestine (periampullary): Clusters (occasional glandular) and discretely lying moderately pleomorphic columnar cells with prominent nucleoli, vacuolated cytoplasm.

Colorectal adenocarcinoma: Tumor cells lying frequently in glandular or palisades, usually columnar; necrotic background.

Neuroendocrine tumor of ileum: Monomorphic small round cells in discrete and loosely cohesive clusters. Cells have salt and pepper chromatin, inconspicuous nucleoli and granular scanty cytoplasm.

Non- Hodgkin's lymphoma: Discrete cells with monotonous population of immature lymphoid cells and scanty cytoplasm. Lymphoglandular bodies in the background.

Endoscopic biopsies were examined by histopathologists who were blind to the cytological findings and the diagnoses. The interpretation of crush cytology of each case was compared with the histopathology, categorised as "malignant" and "non-

malignant". "Suspicious of malignancy" on cytology were also considered as "positive for malignancy" for statistical purposes.

Sample size with justification: As this study of comparative assessment of diagnostic accuracy of crush smear cytology findings with histopathology in evaluation of GI malignancy involves estimation of various proportions by using the formula for sample size calculation for estimating proportion has been used:

$$n = Z^2_{1-\propto/2} P(1-P)/d^{2n}$$

Where n = Minimum sample size, $Z^2_{1-\propto/2}$ = value of the standard normal variant 1-∝/2 for level of significance, P = Anticipated population proportion taken as 0.50, confidence level 100(1-∝/2) = -95%, d = absolute precession required on either side of the population taken as 7.5%. With these values of the input, the minimum sample size required was computed at 171. However, we have achieved a sample size of 180 after eliminating 4 cases for inadequate yield in cytosmear. IBM SPSS Statistics 24.0 of SPSS South Asia Pvt. Ltd. has been used. Distribution by categorical variables like age group, gender and endoscopic findings have been studied by using frequency procedure. The diagnostic accuracy of crush smear cytology with histology as the ultimate method has been done by using diagnostic test evaluation calculator - MEDCALC software (https:// www.medcalc.org/calc/diagnostic_test.php). Sensitivity: probability that a test result will be positive when the disease is present (true positive rate), Specificity: probability that a test result will be negative when the disease is not present (true negative rate), positive predictive value: probability that the disease is present when the test is positive, negative predictive value: probability that the disease is not present when the test is negative and *diagnostic accuracy*: overall probability that a patient is correctly validated.

RESULTS

In the current study, a total of 184 samples of patients with suspicious GI malignant lesions on endoscopic or colonoscopic examinations, were received for cytology and histopathology. Out of those, four samples were excluded because of no cell yield, hence, rest 180 samples were subjected for evaluation. Preponderance of male 63.3% with male: female ratio of 1.73:1. The youngest patient with malignancy was 30 yrs old. The commonest age group for malignancy in our study was 51 to 60 years (Table 1).

The distribution of GI malignancies in different sites is depicted in Table 2. Out of all GI samples received in the department of pathology, it was noticed that gastric growths were the majority (66.67%) followed by colorectal (15.56%), small intestinal (10%), oesophageal (4.44%) and gastroesophageal growths (3.33%).

On crush cytology 89 cases (45.45%) were diagnosed as malignant, one cases reported as suspicious lesions (0.55%) were also considered as malignant, with a sum total of 90 (50% %) malignant cases. On histopathology, 95 (52.77%) patients were diagnosed as malignant lesions. (Table 2). The results were interpreted in Fig. 1 as follows: True positive (TP) - Cytology correctly interprets a case as malignant; True negative (TN) - Cytology correctly interprets a case as non- malignant; False positive (FP) - Cytology falsely interprets a case as malignant; False negative (FN) - Cytology falsely interprets a case as non-malignant; Sensitivity: Likelihood that patient with the disease has positive test results; Specificity: Likelihood that patient without disease has negative results. The cytological diagnoses were correlated with those of clinical and histopathology diagnoses. The results were statistically analyzed. Sensitivity, specificity, positive and negative predictive values, diagnostic accuracy of crush cytology was calculated in comparison with histopathology. All data were compiled, tabulated, compared and analyzed using standard statistical methods. The statistical significance of this categorical data was assessed using SPSS16 software.

The sensitivity (true positive rate) of crush smear cytology was found to be 93.68% with 95% CI 86.76% to 97.65%. On histology, 85 (47.23%) cases and on cytology 90 (50%) cases were diagnosed as non-malignant. Thus, the specificity (True negative rate) was found to be 83.53% with 95% CI 73.91% to 90.69%. A positive likelihood ratio, or LR+, is the "probability that a positive test would be expected in a patient divided by the probability that a positive test would be expected in a patient without a disease". In other words, an LR+ is the true positivity rate divided by the false positivity rate was 5.69 with 95% CI 3.51 to 9.21. A negative likelihood ratio or LR- is "the probability of a patient testing negative who has a disease divided by the probability of a patient testing negative who does not have a disease, was 0.08 with 95% CI 0.03 to 0.16. The positive predictive value i.e. probability that the disease is present when the test is positive was 86.41% with 95% CI 79.71% to 91.14%. The negative predictive value probability that the disease is not present when the test is negative was 92.21% with 95% CI 84.43% to 96.27%. The diagnostic

Table 1: Age and Gender distribution of cases

Age group	Male	Female	Total - n (%)
≤ 40 years	27	15	42 (23.3%)
41-50 years	22	14	36 (20%)
51-60 years	52	22	74 (41.1%)
> 60 years	13	15	28(15.6%)
Total - n (%)	114 (63.3%)	66 (36.7%)	180

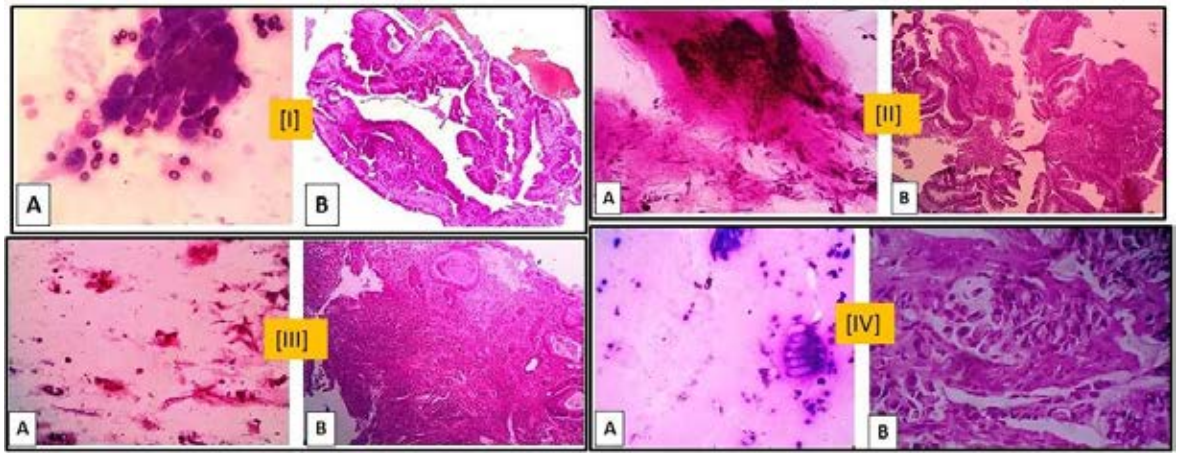

Fig. 1: [I] True positive: A- Cytology: 3D clusters of tumor cells with cellular atypia and in glandular pattern; Necrotic background (features of malignancy) (DQ100X); B- Histology: Adenocarcinoma of colon (H and E100X). [II]True negative: A- Cytology: Benign rectal polyp (negative for malignancy) (H and E 100X); B: Histology: Juvenile rectal polyp (H and E100X) [III] False positive: A- Cytology: Mostly dispersed cells and loose acinar clusters with features of atypia (features of malignancy) (H and E100X); B- Histology: Ulcerative colitis with pseudo polyps (H and E40X) [IV]False negative: A- Cytology: Benign gastric ulcer (negative for malignancy) (DQ100X); B- Histology: Gastric adenocarcinoma, diffuse type (H and E100X)

Table 2: Distribution of lesions as per GI sites of involvement with Cytologic and histopathologic categories

		Cytology			Histopathology	
Site of GI Lesions	No. (%)	Malignant No.	Suspicious No.	Non malignant No.	Malignant No.	Non malignant No.
Oesophageal growth	8 (4.44%)	6		2	6	2
Gastric growth	120 (66.67%)	70	1	49	69	51
Small intestinal (Duodenal and Ilial) Growth	18 (10%)	11		7	11	7
Gastroesop hageal junction growth	6 (3.33%)	4		2	4	2
Colorectal growth	28 (15.56%)	7		21	6	22
Total	180	89 (45.45)	1 (0.55)	90 (50)	95 (52.77)	85 (47.23)

Table 3: Correlation of cytologic diagnosis with the ultimate standard diagnosis on histopathology (Diagnostic accuracy)

	Histopathololgy diagnosis (ultimate Standard method)				
Cytology diagnosis	Malignant (+ve)	n	Non-malignant (-ve)	n	Total
Malignant (+ve)	TP	a = 89	FP	c = 14	a+c = 103
Non-malignant (-ve)	FN	b = 06	TN	d = 71	b+d = 77
Total		a+b = 95		c+d = 85	a+b+c+d = 180
Diagnostic accuracy	TP + TN / N x 100 = 94.82%				

Table 4: Diagnostic accuracy of Cytology diagnosis with Histology diagnosis in Gastric and Colorectal lesions

	Histopathology diagnosis						Total	
Cytology diagnosis	Malignant (+ve)	Gastric n	Colo rectal n	Non-malignant (-ve)	Gastric n	Colo rectal n	Gastric n	Colo rectal n
Malignant (+ve)	TP	a = 63	a = 06	FP	c = 10	c = 02	a+c = 73	a+c = 08
Non-malignant (-ve)	FN	b = 6	b = 02	TN	d = 41	d = 18	b+d = 47	b+d = 20
Total		a+b = 69	a+b = 08		c+d = 51	c+d = 20	a+b+c+d = 120	a+b+c+d = 28
Diagnostic accuracy	Gastric	86.87%						
	Colo rectal	85.71%						

accuracy of test was 94.82% with 95% CI 83.36% to 97.08%. This analysis indicated cytology can be considered as a highly efficient diagnostic tool for GI malignancy. Crush cytology and histopathological findings on biopsy were compared and the diagnostic accuracy of crush cytology for detection of non-malignant and malignant lesions was evaluated taking histopathology as the reference standard test (Table 3).

In the present study, the evaluation of diagnostic efficacy of crush smear cytology findings in comparison to histology (ultimate /gold standard) in detecting the malignant and non-malignant lesions in gastric finding cases has been studied in Table 4. The sensitivity (true positive rate) of crush smear cytology was found to be 91.30% with 95% CI 82.03% to 96.74%. The specificity (True negative rate) was 80.39% with 95% CI 66.88% to 90.18%. The positive likelihood ratio was 4.66 with 95% CI 2.66 to 8.16. The negative likelihood ratio was 0.11 with 95% CI 0.05 to 0.24. The positive predictive value i.e. probability that the disease is present when the test is positive was 86.30% with 95% CI 78.25% to

91.69%. The negative predictive value probability that is the disease is not present when the test is negative was 87.23% with 95% CI 75.87% to 93.69%. The diagnostic accuracy was 86.67 with 95% CI 79.25% to 92.18%. This analysis indicated crush cytology can be considered as a highly efficient diagnostic tool for gastric malignancy as well. Diagnostic accuracy test on esophageal, gastroesophageal junction masses and small intestinal lesions were not done due to their undesirable small sample size.

DISCUSSION

Endoscopy procedure has greatly facilitated the detection of GI lesions for malignancies (cancer) as it helps in direct visualisation of the mucosal lesions, and at the same time it permits the collection of tissue sample for biopsy and cytology to reach at a definitive diagnosis[9,10]. Endoscopic biopsy has been the routine method since long in diagnosis of GI lesions as it is considered as the ultimate standard method[6]. We have undertaken a study considering a cytologic method (crush smear) adopting it as a primary technique in detecting GI malignancies. Crush smear cytology is also useful for diagnosis of GI malignancies. Crush smear cytology is a cheap, easy and readily performed technique that needs only a minute amount of tissue giving a high diagnostic yield and expedites diagnostic work up of various GI lesions and found to be useful as compared to conventional histopathology (mucosal biopsy). Compared with histopathology study, crush preparations require minute amount of tissue and provide rapid diagnosis[11-13]. Crush cytology and histopathology have equivalent diagnostic reliability for malignancy of large intestine, stomach and oesophagus[11,14]. Set side by side with other cytotechniques like brush and imprint (touch) cytology popularised within the last few years, crush smear cytology is found to be superior mainly because of high cell yield and enables visualisaion of malignant cell that stand out amongst the superficial ulcerative debris. Edema and hemorrhages do not significantly affect the quality of preparation. Hence, Crush smear represents the cellular materials from whole of the sample[15].

180 cases of GI lesions were analysed during the period of 2 years by crush cytology and concurrent histopathology for the purpose to verify diagnostic accuracy of malignancy on cytologic method. In our study, there were cytohistologic discordance accounting for 14 false positive and 6 false negative smears. The overlapping features for these discordance were evaluated as follows: False positive cases from gastric lesions were attributed to the following reasons: [i] the non- malignant vacuolated cytoplasm of oxyntic mucosa seen in proton pump inhibitor drug induced gastritis appeared signet ring like cells in cytology which was considered as malignant in cytology. [ii] Smears with dyscohesive atypical epithelial cells in moderate number were reported as malignant but on histopathology those were proved to be inflammatory regenerative atypical changes of surface lining cells non- malignant feature on HP study of stomach[16]. One smear on cytology was reported as suspicious for malignancy cameout as malignancy on HP study. The most reliable criteria to differentiate severe reactive atypia from malignancy are the lack of three dimensional groupings, cell dishesion, single cells, pleomorphism, coarse irregular chromatin and thick irregular nuclear membranes[1]. Those had been attributed to cells regenerating from margins of benign gastric ulcers, because morphologically the distinction between regenerating atypia and malignancy proves to be difficult to assess in cytology and accounted for majority of observation in gastric lesions of our study (Fig. 2)[7].[iii] Resemblance of small enterochromaffin

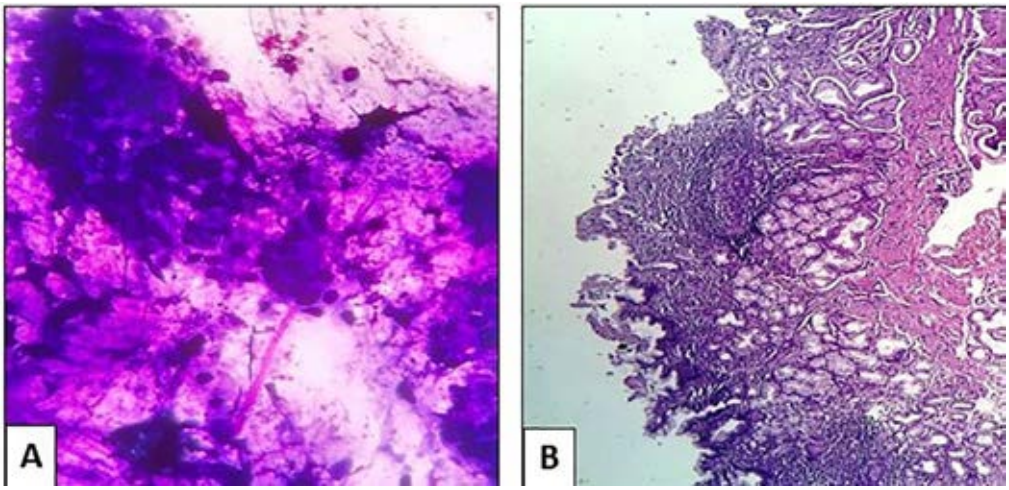

Fig. 2: Suspicious of malignancy: A- Cytology: Loosely cohesive clusters of mildly atypical cells (feature of malignancy) (DQ100X); B- Histology: Margin of Benign gastric ulcer (H and E40X)

like cells in atrophic gastritis (non- malignant) case diagnosed in HP study appeared as diffuse type poorly differentiated mucin poor carcinoma on cytology[17]. [iv] False positive case documented from rectum in crush cytology were the atypical appearing epithelial cells reported as malignant in cytology and those cells atypical appearing cells were regenerating crypt epithelia (non- malignant features) in pseudopolyps of ulcerative colitis confirmed on HP study (Fig. 1)[7]. False negative was attributed to the following reasons: [i] small cells with histiocytic looks predominantly found in scattered manner were reported as chronic gastritis (non- malignant) but on Histopathology (HP) study they were proved to be mucin poor variant of diffuse type carcinoma. [ii] Diagnostic signet ring cells were few and benign lining epithelial cells were evident in the smear , may be because of low cell yield and were considered as histiocytes on cytology (Fig. 1)[7]. [iii] A gastric adenocarcinoma documented on HP was reported as benign ulcerative lesion viewing necrotic debris and no diagnostic cells (non- malignant) on cytosmear , thus resulted in false negative diagnosis.

All lesions both cyto- and HP study were malignant in esophageal, gastroesophageal junction and periampullary duodenal sites though very small sample sizes collected in the study period. Malignancies in esophagus was all found to be squamous cell carcinoma wheras the rest of the GI site lesions were adenocarcinoma satisfying the cellular features. A growth in duodenal mucosa diagnosed as neuroendocrine tumor both in cyto- and HP study. Another ulcerative growth in descending colonic mucosa reported as non- Hodgkins Lymphoma in histology corroborated with the cytosmear diagnosis showed discohesive monotonous atypical lymphoid cells in crush smear[1,8]. Cases of tubular adenoma colon and gastric adenoma diagnosed on histology lined by dysplastic epithelial cells appeared as infiltrating malignant cells in cytosmear[14]. The limitation of cytology in these smears is its inability to distinguish between dysplasia and invasive carcinoma and a high cellularity in a smear may indicate invasion ,but not with certainty. Hence, suspicious for malignancy in smears were marked as malignant in cytology as well[7].

The crush smear diagnostic accuracy (94.82%) on whole of GI lesion malignancies are comparable to study carried out by Desai *et al*.[4] and Buchireddy *et al*.[13] Literature shows these two crush smear cytology studies are conducted on all the lesional sites of gastrointestinal tract. Batra[11] tried with combinations of cytotechniques,including crush smear, on upper Gastric lesions where they have assessed the diagnostic accuracy similar (81.25%) to our study (86.87%) on oesophageal and gastric growths. Colorectal malignancy in our study showed a sensitivity of 95% and specificity of 64.2% and diagnostic accuracy of 89.5% which is verging on study carried out by Chaithra *et al*.[14] and Saha *et al*.[18] These findings are comparable with our study.

The choice of the cytologic procedure i.e brush, crush, or imprint, is the gastroenterologist's prerogative and they usually prefer histopathology diagnoses to cytological diagnosis because histopathology is considered the ultimate diagnosis (gold standard for diagnosis) of malignancy is based on histopathologic evaluation (HPE) of malignancy even if it takes much longer time for processing and reporting. Crush cytology procedure is quick enough process to give the diagnosis of malignancy (cancer) on the same day (within hours) of endoscopy which helps the patient for an early appointment with the oncologist/oncosurgeon and therapeutic decisions can be taken approximately one week earlier. Those suspicious cases detected in our early diagnostic technique ,do not show clearly negative (non-malignant) features, should be kept under surveillance until proved otherwise. High diagnostic accuracy verified with parallel biopsy HP study in our research can draw an inference that crush smear cytology is a cost effective, simple, reliable technique and near-accurate which we performed alongwith biopsy to verify its diagnostic accuracy in endoscopically suspicious GI malignant lesions. However, the drawback of performing crush cytology technique is chances of missing lesions in cases of signet ring cell carcinoma and also mucin poor variant of diffuse type of gastric malignancy where extra care is needed before commenting a smear as negative for malignancy cytologically.

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