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Concordance between Brush Cytology and Histopathology in diagnosing Gastrointestinal Malignancies: Observations from Rural Medical College

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Abstract

Background: Gastrointestinal (GI) cancers remain a critical concern in global healthcare, representing a significant portion of cancer-related complications and fatalities. These malignancies make up roughly 25% of cancer cases worldwide. A concerning trend has emerged, particularly in East Asia, where there is a noticeable increase in the incidence of colorectal cancers among younger individuals. The diagnosis of GI cancers is typically delayed due to the nonspecific nature of symptoms, often leading to diagnosis at advanced stages. Although endoscopic biopsy, followed by histopathological examination, remains the primary method for diagnosing gastrointestinal cancers, brush cytology has become a valuable complementary technique for the early detection of these malignancies.

Aim: The study aims to assess the effectiveness of brush cytology in identifying gastrointestinal cancers and to determine its level of agreement with histopathological results for malignancies in both the upper and lower gastrointestinal tract.

Materials and Methods: A two-year, observational study (both retrospective and prospective) was conducted at a rural medical college in Western

Maharashtra, India. A total of 66 patients suspected of having GI malignancies underwent both video-endoscopic examination and concurrent brush cytology and histopathological sampling. The study focused on patients presenting with lesions in the oesophagus, stomach, and colon. Sensitivity, specificity, accuracy, and cytohistological discordance were evaluated. **Results:** Brush cytology demonstrated an overall sensitivity of 84.7%, a specificity of 71.4%, and an accuracy rate of 83.3% for diagnosis. The study revealed significant discrepancies between cytological and histological diagnoses, particularly in gastroesophageal lesions, highlighting challenges in differentiating squamous cell carcinoma (SCC) from reactive or regenerative changes.

Conclusion: Video-endoscopic brush cytology offers a quick, safe, and reliable complementary method for diagnosing GI malignancies. It enhances diagnostic accuracy when combined with histopathological evaluation, facilitating timely management decisions for patients with suspected malignancies.

Keywords: Brush Cytology, Endoscopy, biopsy, gastrointestinal malignancies, cytohistological concordance.

Introduction

The Global Burden of Diseases report indicates that cancers of the gastrointestinal tract are responsible for 36.2% of deaths related to neoplasms.[1] Typically, patients with gastrointestinal (GI) cancers are diagnosed at advanced stages. Initially, the detection of GI malignancies relied on histopathological examination when suspected clinically and by radiological investigations. The introduction of fibreoptic endoscopy has enabled the collection of cytology samples for diagnostic purposes. Numerous studies have assessed the sensitivity of brush cytology. Some research has shown a sensitivity of 98.03% for upper gastrointestinal lesions[2] and 88% for colorectal malignancies,[3] while other studies have reported considerably lower figures.[4,5] Most of these studies concentrate on either upper or lower gastrointestinal (GI) cancers, with only a few examining the efficacy of brush cytology for both upper and lower GI malignancies.[6-8] This study aims to evaluate the diagnostic utility of brush cytology in detecting cancers throughout the entire GI tract, encompassing both upper and lower regions.

Materials and Methods: This retrospective and prospective observational study was conducted over two years, from January 2022 to January 2024, at a rural medical college in Western Maharashtra after approval by ethical commiitte. Observations from cytology and histopathology were recorded for individuals who underwent diagnostic endoscopy of the upper gastrointestinal tract and colonoscopy to identify potential malignancies. The results of these examinations were documented.

Study population

Inclusion criteria

1. Patients with a presumptive diagnosis of gastrointestinal malignancy are referred for endoscopic evaluation and tissue diagnosis.

Exclusion criteria

1. Diagnosis achieved by alternative procedure eg image-guided biopsy or fine needle aspiration
2. Cases where either cytological or tissue diagnosis was not available

Endoscopy procedure: Patients meeting inclusion criteria underwent an endoscopy procedure. Those patients with upper gastrointestinal (GI) tract malignancy underwent upper GI endoscopy (UGI scope)

and those with suspicion of colorectal malignancy (CRC) underwent colonoscopy.

All patients were evaluated by a skilled gastroenterologist with experience of >1000 upper GI endoscopies and colonoscopies, who performed video-endoscopic examinations of the upper and lower gastrointestinal tracts. Endoscopic examination included the assessment of lesion size, location, appearance, and mucosal fragility. The abnormalities were classified as either ulcerative, polypoidal, or other morphological patterns.

Upper GI endoscopy procedure: After written informed consent, upper GI endoscopy was performed using a standard gastroscope (GIF-H170, Olympus medical systems-Japan) using CV-170 video-processor (Olympus medical systems, Japan) under conscious sedation given by an anaesthetist.

Colonoscopy procedure: Colonoscopy was performed using a standard colonoscope (CFH-170 AL, Olympus Medical Systems, Japan) after a split-dose bowel preparation regimen (polyethylene glycol-based) with or without T Bisacodyl. Bowel preparation quality was assessed using the Boston Bowel Preparation Score (BBPS). BBPS score < 6 was considered as inadequate preparation and the procedure was repeated.

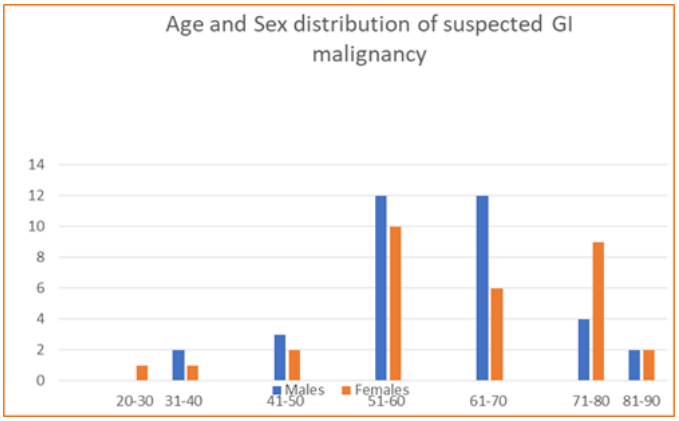
Biopsy and cytology procedure: Brush cytology was taken using a disposable cytology brush. Samples were then spread onto two to three glass slides. Air-dried smears were stained using May-Grünwald-Giemsa (MGG) stain, while alcohol-fixed smears were stained with Hematoxylin and Eosin (H&E) and Papanicolaou (PAP) techniques for detailed cytological evaluation. Endoscopic biopsy samples were obtained using Standard disposable biopsy forceps (Endojaw, Olympus medical systems). Tissue samples were processed immediately after fixation in 10% formalin and subsequently stained using hematoxylin & eosin for routine histological evaluation. Both cytology and histopathological specimens were analyzed independently by a panel of pathologists. The initial cytologic evaluations were categorized into four groups: malignant, suspect neoplastic, benign and indeterminate. Samples classified as indeterminate lacked sufficient cellular content for proper lesion assessment.

Data Analysis: Categorical variables were represented using proportions or percentages. The cytological and histopathological results were compared and categorized as follows:

True positive (TP): When cytology correctly identifies a malignant lesion that was confirmed by biopsy. True negative (TN): When cytology correctly identified a benign lesion that was confirmed as non-malignant by biopsy. False positive (FP): When cytology incorrectly identifies a benign lesion as malignant. False negative (FN): When cytology failed to detect a malignant lesion that was confirmed by biopsy. To assess the efficacy of brush cytology, various diagnostic metrics were calculated, including sensitivity, specificity, positive and negative predictive values (PPV and NPV), as well as overall diagnostic accuracy.

Results

Demography and patient characteristics: Sixty-six patients with suspected GI malignancies underwent both video-endoscopy brush cytology and histopathological examination serving as the gold standard. Graph 1 illustrates the patient cohort which included 35 (53.03%) male and 31 (46.96%) female, with median age 62 years.



Graph 1: Age and sex distribution of suspected GI malignancy.

Clinical symptoms and signs: Symptoms such as difficulty swallowing were the symptoms in patients with advanced oesophageal malignancies, while unintentional weight reduction, anaemia, fatigue, nausea, vomiting, and dark-colored stools were more commonly linked to cancers in the upper gastrointestinal tract. In cases of lower gastrointestinal cancers, patients frequently reported symptoms such as distension and abdominal pain, changes in bowel movements, and the presence of blood in faeces.

Distribution of Malignancies: Of the 66 cases, 58 were histopathologically confirmed as malignant. The most common malignant lesions were located in the colon and rectum (26,44.82%) followed by oesophagus (22, 37.93%), the stomach (8,13.79%), and single case involving duodenum and anal verge each respectively. Table 1 illustrates the distribution of gastrointestinal cancers according to their anatomic location.

Table 1: Distribution of Malignant cases on Biopsy

Site	No of cases Total (%)
Oesophagus	22
Stomach	8
Small Intestine - Duodenum	1
Large Intestine	26
Anal verge	1
Total	58

Sensitivity, specificity, PPV, NPV and diagnostic accuracy of brush cytology in diagnosis of malignancy: The effectiveness of Brush cytology in identifying GI malignancies is evaluated using sensitivity, specificity, and accuracy measurement.

Table 2 shows cytohistological correlation. The cytological findings were in concordance with histopathological results in 50 (75.75%) cases, with 11(16.66%) instances showing discrepancies, primarily in gastroesophageal lesions.

Table 2: Cytohistological comparison

Cytology/ Histology Diagnosis	True - positive TP	False -positive FP	False-negative FN	True -negative TN	Total
Esophagus	18	1	3	3	25
Stomach	5	0	3	1	9
Intestine	27	1	3	1	32
Total	50	2	9	5	66

As noted in Table 3 the overall diagnostic accuracy for brush cytology across all GI malignancies was 83.3%.

Table 3: Sensitivity, specificity and accuracy of brush cytology in diagnosing malignant GI lesions

Site	Sensitivity %	Specificity %	Accuracy %
Esophagus	85.7	75	84
Stomach	62.5	100	66.6
Intestine (Lower GIT)	90	50	87.5
Total	84.74	71.4	83.3

Esophageal Cancer

Among 25 suspected of oesophageal malignancies on endoscopy, 22 were confirmed malignant through biopsy. Of these 22 cases, 19 were identified as squamous cell carcinoma (SCC) and three as oesophageal adenocarcinoma. Cytological examination of two adenocarcinoma cases revealed groups of moderately atypical columnar cells with hyperchromatic nuclei (Fig 1a). Endoscopy of a polypoid proliferative growth (Fig. a) at the middle 1/3 of the oesophagus on cytology showed dysplastic squamous epithelial cells (Fig. b), and biopsy revealed well-differentiated squamous cell carcinoma (Fig. c). False-negative results (3 cases, 12%) were attributed to one adenocarcinoma and two SCC cases.

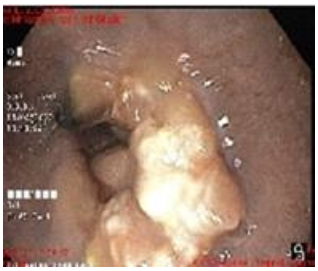


Fig a: Oesophageal Endoscopy- polypoidal growth.

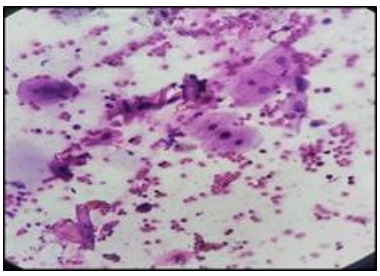


Fig b: Brush cytology- Dysplastic squamous cells.(40X MGM)

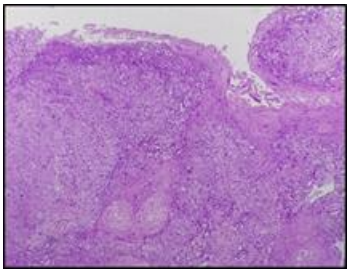


Fig c: Biopsy - Well Differentiated Squamous cell carcinoma (400X HE)

A distal oesophageal adenocarcinoma was missed during cytology, which showed normal squamous cells and a few mildly atypical columnar cells and was reported as suggestive of Barrett's oesophagus (Fig d). However, a biopsy of it revealed well-differentiated adenocarcinoma (Fig e).

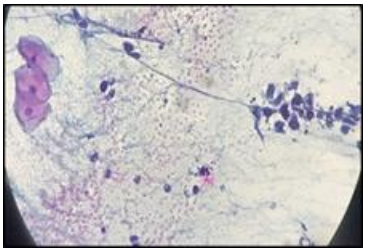


Fig d: Cytology of lower oesophageal growth showing squamous cells and atypical glandular epithelium (40X MGM)

Cytological examination of the plaque-like lesion in the middle third of the esophagus revealed slightly atypical cells, with shapes ranging from round to polygonal, and an increased nucleus-to-cytoplasm ratio. These findings were initially reported as reactive changes. However, subsequent histopathological analysis identified the lesion as a well-differentiated squamous cell carcinoma (SCC) . A single false positive diagnosis reported in another cytology case, where cells displaying atypical features and an increased nucleus-to-cytoplasm ratio were erroneously classified as squamous cell carcinoma

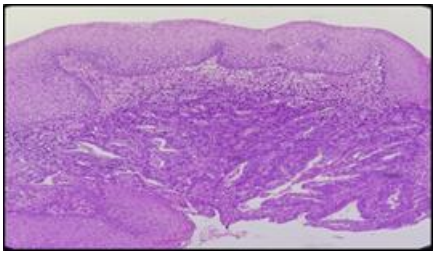


Fig e: Biopsy - Moderately differentiated Adenocarcinoma (400X HE)

(SCC). Subsequent histological evaluation, however, revealed only moderate dysplasia, contradicting the initial cytological assessment

Gastric Cancer: Gastric cancer (adenocarcinoma) was diagnosed in 8(13.63%) patients. Brush cytology showed an overall sensitivity of 62.5% in detecting gastric adenocarcinoma. Cytology smears in a case with thickened gastric wall revealed sheets of benign gastric cells showing the “honeycomb” configuration (Fig f) which on biopsy showed signet ring adenocarcinoma stomach (Fig g) and was missed on brush cytology.

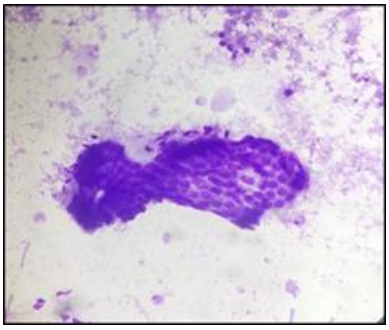


Fig f: cytology showing normal gastric epithelium. (40x MGM)

Colonic Malignancy: Adenocarcinoma of colon was identified in 27 (40.87%) cases. Brush cytology demonstrated a sensitivity of 90% and specificity of 50% in detecting colonic adenocarcinomas, with higher sensitivity for exophytic tumours compared to infiltrating or ulcerative lesions. Some false-negative cases (number and %) were attributed to the sampling of

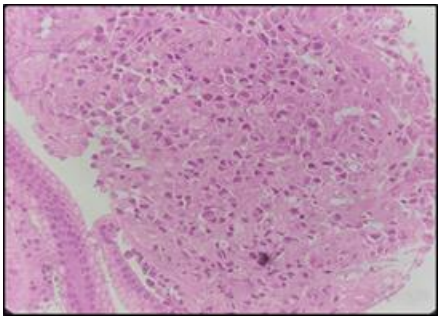


Fig g: Gastric Biopsy - Signet ring Adenocarcinoma (400X H& E stain)

small or necrotic areas, highlighting the challenges in obtaining adequate cellular material for cytological evaluation. Cytology smears of polypoid lesion on colonoscopy (Fig. h) showed clusters of hyperchromatic pleomorphic columnar cells (Fig..i). The lesion on biopsy was confirmed as well-differentiated adenocarcinoma (Fig. j)

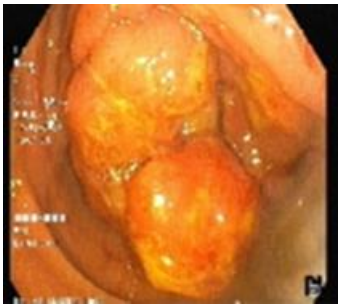


Fig h: Colonoscopy – Polypoidal growth.

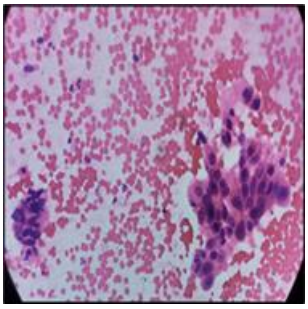


Fig. i: Brush cytology showing atypical columnar cells (40X MGM).

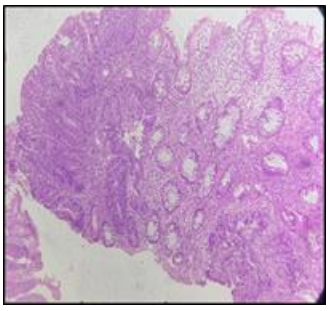


Fig J: Biopsy- Well-differentiated adenocarcinoma (400X HE)

Discussion

Gastrointestinal cancers continue to be a significant global health concern, resulting in over one million fatalities worldwide. In Asia, the occurrence and death rates associated with these malignancies are on the rise. Typically, patients are diagnosed at advanced stages of the disease due to various factors. Nonspecific symptoms, lack of awareness, economic hardship, and misdiagnosis are some of the reasons that contribute to delayed treatment and reduced survival rates.[9]

The patients' ages ranged from 28 to 84 years, averaging 62.5 years. In the study, 60.6% (40/66) of participants were aged between 51 and 70 years, representing the largest age group. This differed from another study where most patients were between 41-60 years old, with a mean age of 58 years.[10] In another study, most participants were over 50 years of age.[8]

Our study found that both genders were similarly affected, with a male-to-female ratio of 1.12:1. The males were 35 and the females were 31 years old. This result indicates a slightly higher prevalence in males compared to other studies.[6,8]

Kobayashi introduced brushing cytology in 1964.[11]

The overall accuracy measures for brush cytology in identifying malignancies within the upper and lower gastrointestinal tract are presented in Table 4. These measures encompass sensitivity, specificity, positive predictive value, and negative predictive value. The results shown are consistent with those observed in similar research studies.

Numerous studies in the literature have focused on brush cytology for upper GI malignancies (Table 4), reporting diagnostic accuracy rates between 78 % and 99.1 %.

Table 4: Comparison of sensitivity, specificity, and diagnostic accuracy of brush cytology of various studies

Author	Lesions	Accuracy%	Sensitivity%	Specificity %
Kasugai et al. (1978) [12]	oesophagus	97	NA	NA
	Cardia	78	NA	NA
	Stomach	78	NA	NA

Young J et al. (1980) [13]	Lower oesophagus and cardia	82	NA	NA
Cook et al (1988) [14]	Stomach	NA	85.1	96.8
Shroff C et al (1988) [15]	Upper GI	97.1	NA	NA
Zargar et al. (1991) [16]	Oesophagus and Gastric	87.9	NA	NA
Vidhyarthi et al (2008) [2]	Upper GI	NA	98.3	81.11
Karmarkar et al (2013) [17]	Upper GI	83.78	NA	NA
Kaur et al (2016) [4]	Upper GI	82.37	83.45	80.95
F. Muniraj et al(2016)[18]	Upper GI	99.1	100	96.4
Present Study	Esophagus	84	62.5	100
	Stomach	66.6	90	50
Geramizadeh B et al (2003) [3]	colorectal	NA	88	96
Brouwer R et al (2009) [5]	Colorectal	NA	88.2	94.1
Tatomirovic Z et al (2017) [19]	Colorectal	89.2	87.9	78.3
Present Study	Intestine (Lower GIT)	87.5	90	50

Researchers have mainly focused on the utility of brush cytology either for upper gastrointestinal (GI)[2,4,12-18] or colorectal lesions.[3,5,19] Research on brush cytology (Table 5) encompassing both upper and lower gastrointestinal cancers is scarce.[6-8]

Table 5: Accuracy metrics of brush cytology in the entire GIT

Author	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Jasim M, Al-Diab et al [7]	91.3%	93.3%	84.6%	96.5%
S.Ojha et al [8]	78.07%	97.7%	98.9%	62.7%)
Tyagi [6]	62.7%	94%,	91.4%	71.2%
Present Study	84.7%	71.4%	96.1%	35.7%

The study found that brush cytology had an 83.3% accuracy rate in identifying malignant GI lesions. Among the patients with suspected oesophageal malignancy, dysphagia was the primary complaint. Out of 25 cases, cytology results showed four positive for malignancy, thirteen suspicious for malignancy, five negative, and three were reported as inadequate. One case deemed positive by brush cytology revealed severe dysplasia on biopsy, necessitating a repeat biopsy to exclude sampling error on biopsy. Histopathology results indicated squamous cell carcinoma in 22 out of 25 oesophageal biopsies, with two cases in the lower third of the oesophagus diagnosed as poorly differentiated adenocarcinoma. Cytological evaluation as a false negative can be explained by the challenges in differentiating between carcinoma and atypia due to regeneration or repair, particularly for pathologists with limited experience. Similar high false-negative rates have been reported in previous studies.[20,21] Among the eight cases of gastric adenocarcinoma confirmed by histopathology, cytology accurately identified malignancy in four cases and one case was reported as suspicious for malignancy. Out of four cases reported as negative on cytology, one was true negative and did not

show malignancy on histopathology. Signet ring adenocarcinoma was not diagnosed on brush cytology as the lesion had less mucosal involvement. One case of well to moderately differentiated adenocarcinoma was misinterpreted as regenerative changes due to the presence of an ulcerative lesion observed during endoscopy. The remaining case, reported as negative on cytology, primarily exhibited necrotic material and a few clusters of gastric mucosa in the cytological examination. Our research revealed that brush cytology was less effective in identifying gastric carcinoma compared to findings from other investigations.[4,13,14]

Neoplasms of the small intestine constitute fewer than 5% of all tumors found in the gastrointestinal tract. and are thought to manifest earlier than other GIT tumors. In our research, we identified two cases of malignancies: one in the duodenum and another in the cecum, both demonstrating 100% sensitivity and specificity. These cases were confirmed through both cytology and histology. Our findings of a limited number of small intestinal malignancies with high sensitivity and specificity using brush cytology align with other studies in the field.[2,6]

In our study, brush cytology yielded negative results in 14.2% of colonic biopsy cases. Limited research exists regarding the cytohistological correlation of colonic biopsies. Other studies have reported slightly higher percentages of false-negative cases, at 18.5% and 23.5%.[6,8] Distinguishing between adenocarcinoma and high-grade dysplasia in colonic tumors through brush cytology is not always feasible. Three false-negative cases on cytology exhibited mild nuclear atypia in two instances and insufficient material in one. Cytology smears classified as suspicious contained unambiguous malignant cells that were confirmed malignant on biopsy, with some displaying characteristics of adenomatous polyp with moderate to severe dysplasia and invasion. Consequently, patients with suspicious cytological reports and negative biopsies should undergo further evaluation, and additional biopsies are recommended after considering clinical and endoscopic findings. In our investigation, all cases deemed suspicious through cytology were confirmed malignant by biopsy, resulting in zero false-positive cases.

In a study of 80 colonic malignancy cases, G H Yu et al noted that high-grade dysplasia poses a diagnostic challenge, leading to cytohistologic discrepancies.[19] Their research revealed that samples from histologically confirmed adenocarcinoma typically exhibited more pronounced alterations in nuclear polarity, nuclear pleomorphism, membrane irregularities, and chromatin patterns compared to those from histologically verified adenomatous or inflammatory lesions. The researchers identified that sampling an adenoma with high-grade dysplasia was the most probable cause of false-positive diagnoses in this context. The present study reported a sensitivity of 90% and a specificity of 50% for colonoscopic brush cytology. The accuracy of detecting colonic malignancy is 87.5%, which aligns with findings from other studies.[4]

In our study, cytohistological discrepancies arise from several factors: insufficient representative samples, inflammation obscuring the cellular details, ulceration at the tumor site, and misclassification of atypical cells as either benign or malignant.

This study demonstrates that brush cytology, when used in conjunction with histopathology, significantly improves the diagnostic accuracy for gastrointestinal malignancies. The sensitivity and specificity of brush cytology varied by tumor location and histological type,

with the highest concordance observed in esophageal and colonic adenocarcinomas. However, challenges in diagnosing certain malignancies, such as gastric and esophageal adenocarcinoma or signet-ring cell carcinoma, were evident, particularly when lesions had subtle or non-exophytic features. Small sample size is the limitation of the current study.

There are limitations of brush cytology in diagnosing infiltrative tumors. Accuracy varies by tumor type. Tumor growth patterns significantly impact the accuracy of brush cytology compared to histopathology in several ways: Brush cytology tends to be less accurate for submucosal and infiltrative tumors compared to more superficial lesions. In one study, aspiration cytology was significantly better than brush cytology for submucosal tumors (92.9% vs 7.1% accuracy) and infiltrative malignancies (95.8% vs 90.1%)[16]. This is likely because brush cytology primarily samples superficial cells, while submucosal and infiltrative tumors may not exfoliate as readily. Brush cytology appears to be particularly challenging for ulceronecrotic malignancies, with one study showing only 36.4% accuracy compared to 90.9% for aspiration cytology. In conclusion, the variability in concordance rates between brush cytology and histopathology is influenced by tumor characteristics (such as growth pattern and size), location, accessibility, and the specific cytology technique employed. These factors should be considered when interpreting cytology results and determining the most appropriate diagnostic approach for different malignancies.

Inter-observer variability also impacts the accuracy. Standardised criteria for cytological evaluation can enhance diagnostic accuracy and reduce inter-observer variability.

Demonstration that microRNAs are readily detectable in brush cytology specimens obtained during ERCP, and have the potential to help the cytological diagnosis of pancreatobiliary malignancy.[22] Detection of microRNA on brush cytology specimens may provide a potential avenue for the prevention and early detection of gastric and colorectal carcinomas after passing the hurdles of feasibility, cost-effectiveness and validation.

Conclusion

Brush cytology is a valuable adjunctive tool for diagnosing gastrointestinal malignancies, particularly when combined with video-endoscopy. It significantly enhances diagnostic accuracy, enabling timely and more informed clinical decision-making. However, careful consideration should be given to its limitations, particularly in detecting deeply infiltrating or mucinous tumors, and it should be used in conjunction with histopathological examination for the most reliable diagnosis.

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