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THE VALIDITY OF RAPID INTRAOPERATIVE AND PREOPERATIVE CRUSH SMEAR CYTOLOGY IN DETECTION OF MALIGNANCIES OF THE GASTRO INTESTINAL TRACT, GALL BLADDER AND THE BREAST.

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ABSTRACT **Aim of study:** To study the reliability of rapid crush smear pre-operative (pre-op) and intra-operative (intra-op) cytological diagnosis in detection of malignancies of gastrointestinal tract (GIT), breast and gall bladder lesions. An early intra-op and pre-op cytological diagnosis is an important diagnostic aid and often considered comparable to histopathological examination (HPE).

Material & Methods: Crush smear preparations of biopsies obtained from 92 patients for an early pre-op and rapid intra-op diagnosis were studied. The diagnosis provided was compared with histopathology on same tissue done later. Endoscopic biopsies from 58 suspicious gastrointestinal (GI) lesions were labeled as pre operative biopsies. In addition, 34 consecutive 'suspicious for malignancy intra operative biopsies' (5 from the GI tract, 11 and 18 were from breast and gall bladder lesions respectively) were subjected to a rapid cytological diagnosis. Reliability of the cytodiagnosis in terms of specificity, sensitivity and predictive values was studied by subjecting the same biopsies to the gold standard histopathological examination.

Results: The sensitivity, specificity, positive and negative predictive values in all the cases altogether were 94.2%, 100%, 100% and 87% respectively. False negative results were obtained for a case of GI biopsy with Non Hodgkin's lymphoma, lesions with predominant necrosis and well differentiated adenocarcinomas. There were no false positives results.

Conclusion: This study emphasizes rapid pre op and intra op cytomorphodiagnosis of squash preparations as a quick, simple, efficacious, inexpensive and a useful adjunct to conventional, time consuming, biopsy reporting of malignancies of the GI tract, breast, and gall bladder.

KEYWORDS : Crush cytology, rapid diagnosis, malignancy

Introduction

Histological study of biopsy specimens is mandatory for an accurate diagnosis of non neoplastic and neoplastic lesions. However the limitation of histology is the longer time required for processing. Although a frozen section of the tissue specimens is considered one of the best modalities for a rapid intra operative diagnosis, this facility, besides involving use of expensive equipment and technical expertise, is not present in many hospitals.

Cytological study, as seen today provides an accurate reflection of many pathological processes. Many workers have tried diagnosing malignancies of the gastrointestinal tract (GIT) based on cytology.[1-9] Lavage studies and wash outs, although enable a large surface area to be sampled, these are time consuming and do not give specificity to the exact site of the sample. Biopsy sampling, especially under direct visioning of the clinician has been found to be superior in the detection of submucosal spreading signet ring carcinoma of the stomach, primary gastric lymphoma and stromal tumors. Study of crush smears of the GI biopsy has been reported to be of immense benefit as a diagnostic modality.[7] Reports of utilizing this diagnostic modality for a rapid intraoperative and an early preoperative diagnosis for lesions of the gastrointestinal tract (GIT), breast, and gall bladder are limited. Crush smears prepared from fresh biopsy tissue specimens received preoperatively (pre-op) as well as intra operatively (intra-op), enables an immediate diagnosis providing vital leads for the physician to plan further prompt treatment.

The present study was conducted to evaluate and compare immediate crush smear cytodiagnosis with histology in the diagnosis of pre-op and intra-op malignant lesions and to establish the reliability of crush smear cytology alone for an early and accurate diagnosis of malignancies**.**

Materials and Methods

This prospective cross sectional study was conducted in a 600 bedded tertiary care center from January 2010 to August 2014. A total of 92 consecutive clinically `suspicious for malignancy' biopsy specimens were subjected to a crush smear cytology preparation. Amongst these, 58 were pre-op endoscopic GI biopsies. In addition, 34 biopsies labeled as intra- op specimens were consecutive `suspicious for malignancy biopsies at surgery. Five of the intra-op biopsies were of the stomach and duodenum, 11 & 18 were from the breast and gall bladder respectively. The fresh biopsy tissue was immediately transferred onto a petri dish. After a gross examination, a tiny representative bit was bitten off from the biopsy tissue, crushed and smeared between two slides. Alternatively, for more solid lesions the surface of the tissue was scraped with a fresh blade and this was subjected to a crush smear preparation. The smears were air dried and stained with Leishman stain, as well as wet fixed for a rapid Papinicolaou stain.

The crush smears were examined and an immediate diagnosis based on the crush smear cytology findings was then given with particular attention to the benign or malignant nature of the lesion. The remaining biopsy tissue was subjected to a histopathological processing as done for routine biopsies, followed by 5 micron sections and staining with hematoxylin & eosin. Special stains were used as and when necessary. Histological examination was taken as the gold standard. The sensitivity, specificity, negative predictive value and positive predictive value were calculated comparing crush cytology diagnosis with histopathology.

Results

A total of 92 biopsy tissue specimens were subjected to a rapid crush smear cytodiagnosis. Out of these, 63 were GI tract biopsies. Five of these were received for an intra-op diagnosis to rule out malignancy. The rest of these were pre- op GIT (58) endoscopic biopsies. The results of crush smear cytology and histology at each site of the GIT have been tabulated and compared in **Table 1**

Oesophagus There were 22 endoscopic biopsies & 21 of these were positive for malignancy. The majority of these were moderately to well differentiated squamous cell carcinomas. Two were reported as poorly differentiated carcinomas and all these were biopsy tissues from the gastro oesophageal junction. There were no false positive or false negative reports. The case reported as benign was a candidial

oesophagitis in a renal transplant patient on immunosuppressive therapy.

Stomach: A total of 17 gastric biopsies were received, out of which 15 were endoscopic biopsies and 2 were for urgent intra-op diagnosis. Benign lesions were seen in five amongst the total of 17 biopsies (all five as non specific inflammatory). Ten were diagnosed as positive for a moderately differentiated adenocarcinoma, one was diagnosed as a poorly differentiated carcinoma and another was a gastric non Hodgkin's lymphoma (NHL). On correlating with the histopathological diagnosis there were no false positive. The biopsy diagnosed as NHL was diffuse large B cell Non Hodgkin's lymphoma in a 70 year old male. One of the lesions diagnosed as negative on cytology was found to be a false negative on HPE. The gastrectomy specimen of this patient revealed a large gastric ulcer (3x3 cm) on the greater curvature with foci of severe dysplasia with intra mucosal invasion. Accompanying severe degree of inflammation was present along with the superficial ulcer.

Duodenum. A total of 11 duodenal biopsies were subjected to a crush smear cytology preparation. Of these 8 were pre-op specimens and 3 were received intra-op for a rapid cytological diagnosis. The intra-op cases were from ulcerated suspect lesions from the peri- ampullary region. Number of lesions diagnosed positive for malignancy on cytology were 2. Of these, one was a moderately differentiated pancreatic duct adenocarcinoma. This smear contained crowded monolayered sheets with moderately tall pleomorphic columnar cells with hyperchromatic nulclei exhibiting palisading at the edges. A microglandular pattern in smaller aggregates could be appreciated in most of the cases. Another one was a neuroendocrine carcinoma wherein a dispersed cell population of monomorphic cells with small amounts of cytoplasm was seen (Fig 1). The nuclei were round to oval with a stippled nuclear chromatin and inconspicuous nucleoli. Also seen was a mild degree of pleomorphism, and a considerable mitotic activity. The presence of accompanying necrosis in some smears helped clinch the diagnosis of a carcinoma. Nine were reported as inflammatory and benign in nature on cytology. There were no false positives, however, two were false negative cases. One of the false negative cases was a well differentiated ampullary duct adenocarcinoma; on reviewing the cytology slide reported as inflammatory it was found to show columnar epithelial cells exhibiting mild atypia in a background of necrosis and acute inflammation and this cytomorphology was interpreted as an ulcerative inflammatory lesion with regenerative atypia (Fig 2). The other was found to be a case of Non Hodgkin's Lymphoma (mucosa associated lymphoid tissue lymphoma). Cytomorphology of the neoplastic small lymphocytes was found to be rather bland even on review of the cytology slides. One of the benign cases comprised of a neoplasm showing a moderately cellular spindle cell proliferation possessing bland cytomorphological features A diagnosis of a gastrointestinal stromal tumor (GIST) of benign nature was given intra-operatively (Fig 3). On HPE, this was diagnosed as GIST smooth muscle type. It was a 2.5 cm nodule expressing positivity for C-kit and smooth muscle actin.

Lower GI tract. Crush smear cytology preparation were done for 13 colorectal biopsies. All of these were endoscopic biopsies. Four of the colorectal crush preparations were reported as benign inflammatory smears and nine were found to be malignant lesions. Most of these were adenocarcinomas. One biopsy from the anal canal was reported as a poorly differentiated carcinoma which on histopathology showed a basaloid pattern. One of the lesions reported as benign was found to be a tubular adenoma with foci of severe dysplasia and evident submucosal invasion (focal carcinoma). Hence although there was no false positive case one proved to be a false negative report.

Gall Bladder. Total 18 cases of gall bladder tissue were received for intra-op cytodiagnosis. Out of these 8 were found to be moderately differentiated carcinomas (Fig 4). All the other 10 were reported as benign. One of the cases was a suspicious polyp of the gall bladder. The crush smear exhibited a benign cytomorphology and was reported as such. On HPE, this polyp was found to be a tubular adenoma with no evidence of dysplasia. There were no false positive or false negative cases.

Breast. Eleven biopsy tissues were received for intra-op cytodiagnosis to rule out malignancy. Of these, nine were reported as malignant on a crush smear cytology and 2 were found to be benign. On HPE, the malignant lesions were found to be invasive ductal cell carcinoma (NOS). The benign lesions were diagnosed as fibroadenoma and a juvenile fibroadenoma. The results of crush smear cytology and subsequent histopathology of the intra- op gall bladder and breast biopsies for detection of malignancy have been tabulated and compared in **Table 2.**

Discussion

Cytodiagnosis is most useful both at a preoperative as well as intra-op stage to expedite early diagnosis, treatment and definitive surgery. Crush cytology has been used extensively in examining central nervous system lesions. Overall, in all the cases included in this study, a sensitivity of 94.2% and specificity of 100 % was obtained. The positive predictive value was 100% and negative predictive value was 87 %.

The GI biopsy cytodiagnosis were found to be 100% specific and 92.3% sensitive. The positive predictive value was found to be 100%, although the negative predictive value was 79%. A false negative diagnosis was obtained in smears which showed predominantly necro-inflammatory features and associated mild degree of dysplasia. Young et al found biopsy crush smear cytology with a sensitivity of 100% to be the most accurate technique for the diagnosis of malignancy of the lower end of the esophagus and the cardia as compared to biopsy histology which had a sensitivity of 89%.[1] Sharma et al [2] have obtained a cytological diagnostic accuracy of 96.3% and 95% respectively for esophageal lesions.[2] Reynolds et al found that in the case of esophageal biopsies cytology was positive in 84% of cases, biopsy in 86%, and combined specimens (cytology and biopsy) in 94%.[3] In our study only 4.5% of the lesions were benign and 95 % of the biopsies received as clinically suspect were positive**.** The biopsy results provided a 100% sensitivity and specificity with no false positive and false negative cases**.** Chambers et al reported that combined endoscopic biopsy and cytology was more sensitive (96%) than biopsy alone (90%) in making the initial diagnosis.[5]

Waldren and Young et al also achieved a high degree of reliability in gastric lesions.[1,9] A high grade lymphoma could be easily diagnosed but an intermediate grade type was difficult to differentiate from associated inflammation. The same has been reported by Kline et al.[10] Biopsies from the periampullary region were most difficult to diagnose. This was seen because of the well differentiated nature of the lesion and lack of architectural details on cytological examination. The same cytological dilemma which has been reported by cytopathologists.[10] A neuroendocrine carcinoma and even a GIST could be diagnosed amongst these cases. Amongst the colorectal biopsies**,** the majority were successfully diagnosed on cytological alone. Exact typing into a basaloid carcinoma was possible only on subsequent histomorphology. A false negative report for a tubular adenomas signifies the importance of architectural details which can be discerned on histology alone as has been reported earlier.[11,12]

There have been reports of the diagnostic utility and even the superiority of intra-op cytomorphology over frozen section diagnosis.[13-15] This study also confirms the diagnostic utility of an intra-op cytodiagnosis of breast lesions and report a 100 % sensitivity and specificity. However diagnostic dilemmas of in situ lesions and the exact histological typing and prognostication would necessitate a histological examination.

Gall bladder cytology has only been reported on fine needle aspiration cytology samples (FNAC). We in this study emphasize the efficacy of a rapid intra op diagnosis to rule out malignancy. Gall bladder lesions are many times inaccessible to a definitive FNAC procedure and a fair number of them remain clinically suspect till the time of surgery. A intra op immediate cytodiagnosis has been found to be very useful to rule out malignancy and hence plan the extent of surgery.

Conclusion

Crush smear cytology from biopsy specimens has been found to be a highly sensitive and specific technique for identification of carcinomas of the GI tract, gall bladder and breast lesions. It is easier, quicker and cheaper to prepare 4-5 crush smears before fixation of each biopsy for routine histological diagnosis and can replace a frozen section preparation for a per op diagnosis in most cases. This technique which does not require any additional effort or equipment may add to the diagnostic yield besides expediting the workup. Histology is however essential for assessing invasion and for tumor grading and typing. The

technique of squash cytology is a useful adjunct to conventional biopsy hence crush cytology and histology are complimentary and both should be utilized together.

Table 1 Comparison of the results of crush smear cytology and histology in the diagnosis of malignancies of Gastrointestinal tract.

Site	Crush cytology diagnosis	Histopathological Diagnosis		Sensitivity	Specificity	PPV	NPV
		Benign	Malignant				
Oesophagus (n=22)							
Benign	1	1	0	100%	100%	100%	100%
Malignant	21	0	21				
Stomach (n=17)							
Benign	5	4	1	92.3%	100%	100%	80%
Malignant	12	0	12				
Duodenum (n=11)							
Benign	9	7	2	66.7%	100%	100%	77.8%
Malignant	2	0	2				
Lower GI Tract (n=13)							
Benign	4	3	1	90.9%	100%	100%	75%
Malignant	9	0	9				
Total (n=63)							
Benign	19	15	4	92.3%	100%	100%	79%
Malignant	44	0	44				

PPV - Positive Predictive value
NPV - Negative Predictive value

Table 2 Comparison of the results of crush smear cytology and histology in the diagnosis of malignancies of the gall bladder and breast and of the total cases investigated.

Site	Crush cytology diagnosis	Histopathological Diagnosis		Sensitivity	Specificity	PPV	NPV
		Benign	Malignant				
Gall Bladder (n=18)							
Benign	10	10	0	100%	100%	100%	100%
Malignant	8	0	8				
Breast (n=11)							
Benign	2	2	0	100%	100%	100%	100%
Malignant	9	0	9				
Total cases investigated (n=92)							
Benign	31	27	4	94.2%	100%	100%	87%
Malignant	61	0	61				

PPV - Positive Predictive value , NPV - Negative Predictive value

Fig 1: Crush smear cytology of periampullary neuroendocrine carcinoma (Leishman Giemsa x200)

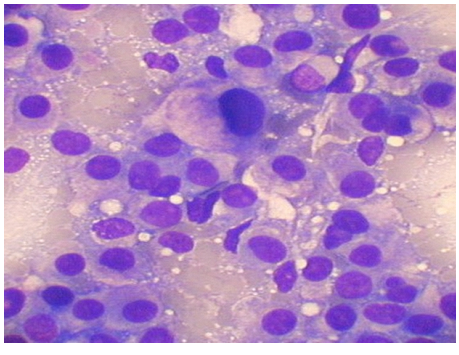


Fig 2 :Crush smear cytology of periampullary adenocarcinoma with accompanying neutrophilic inflammation (PAP x 200)

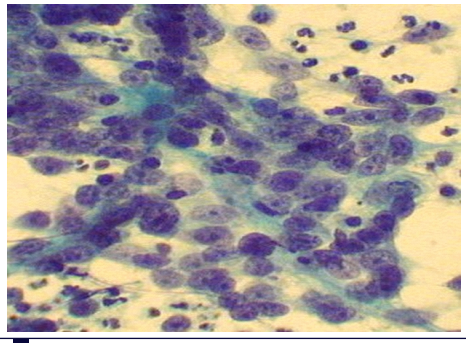


Fig 3 : Crush smear cytology of Gastrointestinal stromal tumor of the duodenum (PAP X400)

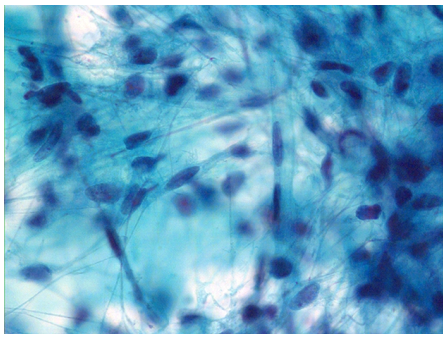
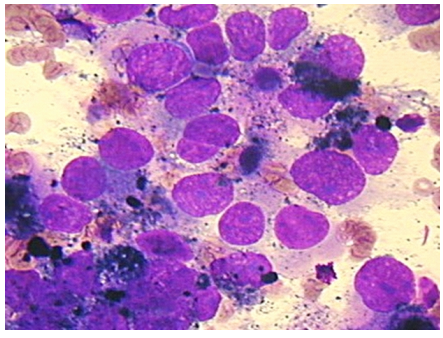


Fig 4: Crush smear cytology of adenocarcinoma gall bladder (Leishman stain x400)



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