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Original Article

Imprint cytology: A diagnostic aid in interpretation of upper gastrointestinal endoscopic biopsies

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Abstract

Context: Although major advances have occurred in the cytological diagnosis of various organ pathologies, gastrointestinal (GI) cytology has not gained popularity and is only occasionally practiced in the form of brushings and washings. Biopsy touch imprint cytology (IC) is a rapid, simple and inexpensive method of diagnosis of upper GI lesions. Thus, a study to show a correlation of IC and standard histopathology will allow the procedure to be performed routinely as adjunct to histopathology. **Aims:** To correlate results of imprints made from upper GI biopsies with histopathology in case of suspected upper GI lesions and to compare detection of *Helicobacter pylori* by IC and histopathology in suspected *H. pylori* infection. **Subjects and Methods:** One hundred and ten cases of upper GI endoscopic biopsies were included in the study. Touch imprints were made from the biopsies, stained and studied. The same tissue was put in 10% formalin for histopathological processing. Results of IC were compared with histopathology. **Statistical Analysis Used:** Chi-square/Fisher exact test were used to find out significance of study parameters. **Results:** Sensitivity and specificity of IC for neoplastic lesions were 94.3%, 100% for esophageal lesions; 88.2%, 97.14% for gastric lesions and 100%, 100% for duodenal lesions respectively, which correlated with other studies. Imprint smears for *H. pylori* had sensitivity of 80%, which was higher than that of brush cytology. **Conclusions:** IC is a valuable diagnostic tool and can be routinely applied as an adjunct to histopathological examination in the diagnosis of GI lesions.

Key words

Endoscopic biopsy, imprint cytology, upper gastrointestinal cytology

Introduction

Endoscopic biopsy is an essential part of the evaluation of gastrointestinal (GI) pathology. In the diagnosis of upper GI lesions, histopathological examination (HPE) is considered gold standard but is time-consuming compared to cytology. Most gastroenterologists and patients would like an immediate opinion regarding the adequacy of biopsy and nature of the lesion. Imprint cytology (IC) is a simple, rapid and cost effective method, though rarely practiced.[1] Sensitivity and specificity of IC recorded in various studies range from 95% to 100%.[2,3] IC is useful in diagnosing *Helicobacter pylori* infection which is a premalignant, but treatable condition.[4]

Thus, a study to show a correlation of results of IC and HPE will be helpful and may allow the procedure to be performed more often.

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Subjects and Methods

Totally 110 patients with upper GI symptoms, who underwent endoscopic biopsies from esophagus, stomach and duodenum (up to Ampulla of Vater) were included in the study. Recruitment of subjects into the study was done over a period of 2 years. Six biopsies with inadequate material and slides showing crush artefacts were excluded.

Four to six biopsies were taken from lesions and touch imprints of the biopsies were made from fresh biopsy by gently

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rolling the tissue on glass slides using needles and applying gentle pressure at intervals. A minimum of four imprints were made, fixed in 95% alcohol and stained with Papanicolaou and Hematoxylin and Eosin stains. In cases of suspected *H. pylori* gastritis, an additional air-dried imprint smear was made and stained with May-Grunwald-Giemsa (MGG) stain. The same biopsy tissue was put in 10% formalin for fixation and routine histopathological processing was done. Sections were cut at 5 μm thickness and stained with Hematoxylin and Eosin.

On cytology, lesions were categorized as "unsatisfactory" when imprint slides showed low cellularity or cells obscured by blood/mucous; "negative for malignancy" when slides showed no atypia or mild atypia in the presence of inflammatory cells; "suspicious of malignancy" when borderline atypia was seen in the presence of low cellularity;[5] "Positive for malignancy" in presence of hypercellularity, nuclear irregularity, macro-nucleoli, high N: C ratio, signet ring cells, tumor cannibalism.[6]

On histopathology, lesions were categorized as "negative for any pathology", "inflammatory lesion," "dysplasia" and "positive for malignancy."

The imprint smears and the biopsies were seen by the same pathologist, though blinded to cytology report while interpreting the biopsy to avoid bias. All slides were reviewed by a second pathologist.

Statistical analysis

The results of IC were then correlated with histopathology.

Chi-square/Fisher exact test were used to find the significance of study parameters (*P* value) with 95% confidence intervals.

Results

The study included 110 endoscopic biopsies including 45 (40.9%) esophageal, 52 (47.3%) gastric and 13 (11.8%) duodenal biopsies. 34 (75.6%) esophageal lesions, 17 (32.7%) gastric lesions and 5 (38.5%) duodenal lesions were neoplastic.

Most of the patients who underwent endoscopy were in the fifth to seventh decades of life, with a mean age of 55 years.

A male preponderance was seen among patients with an M: F ratio of 2.1:1. Most of the neoplasms occurred in males with M: F ratio of 2.6:1, 3.3:1 and 0.7:1 for esophagus, stomach and duodenum, respectively.

Dysphagia was the most common indication for endoscopic evaluation in esophageal lesions, acid peptic disease (APD) for gastric lesions and jaundice and APD for duodenal lesions.

The sensitivity, specificity and diagnostic accuracy of IC were 94.29, 100, 95.56% for esophageal lesions, 88.24, 97.14, 94.23% for stomach, and 100, 100 and 100% for duodenal lesions.

Distribution of imprint cytology findings with corresponding histopathological examination [Table 1].

Distribution of histopathological examination report of patients studied [Table 2].

Analysis of imprint cytology versus histopathological examination for malignancy-site wise [Table 3]

Out of 65 biopsies from the stomach (52) and duodenum (13), *H. pylori* was detected in 9 (17.3%) cases of stomach and 1 (7.7%) case of duodenum on HPE. *H. pylori* was identified on imprint slides in eight cases (seven gastric and one duodenum). One case was associated with adenocarcinoma.

Analysis of imprint cytology versus histopathological examination for *Helicobacter pylori*-site wise [Table 4]

The sensitivity, specificity and diagnostic accuracy of IC for *H. pylori* were 80, 100, and 96.9%. Chi-square/Fisher exact test was applied and $P < 0.001$ indicating a significant association.

Discussion

Gastrointestinal cytology is still in its early stages of development, and only a few studies have described the role of brush cytology and touch IC in interpretation of upper GI biopsies.

For esophageal lesions, the sensitivity, specificity, and diagnostic accuracy of IC were comparable with results of Sharma *et al*., but lower than those of Mysorekar *et al*. and Young *et al*.[2,3,7] The reasons for lower sensitivity were two false negative cases; one case was unsatisfactory on IC due to large areas of necrosis and showed squamous cell carcinoma on HPE. One case that was reported negative for malignancy on IC, turned out to be squamous cell carcinoma on HPE due to subepithelial location of the tumor, which was missed on imprint.

One case showed columnar metaplasia [Figure 1] in the absence of goblet cells. Though "no goblet no Barrett" was the rule,[8] the presence of columnar metaplasia along with endoscopic confirmation is now considered sufficient for a diagnosis of Barrett esophagus.[9]

Twenty-six smears were labeled as "positive for malignancy". Most smears showed good cellularity. Squamous cell carcinoma was identified by presence of pleomorphic squamoid cells with orangeophilia demonstrable on Pap stain with tumor diathesis [Figure 2]. One case on imprint smear showed adenocarcinoma.

Table 1: Distribution of imprint cytology findings

Imprint cytology report	Esophagus		Stomach		Duodenum		Total
	Number (%)	Corresponding HPE reports	Number (%)	Corresponding HPE reports	Number (%)	Corresponding HPE reports	
Unsatisfactory	01 (2.2)	Poorly differentiated carcinoma with large areas of necrosis	01 (1.9)	Chronic gastritis	00		2
Negative for malignancy	12 (26.7)	6-no significant pathology 4-nonspecific inflammation 1-columnar metaplasia 1-poorly differentiated carcinoma with predominant sub epithelial location	35 (67.3)	30-inflammation 3-no significant pathology 2-adenocarcinoma	08 (61.5)	8-chronic inflammation	55
Suspicious of malignancy	06 (13.3)	4-malignant 2-dysplasia	02 (3.8)	1-malignancy 1-inflammation with reactive atypia	03 (23.1)	2-adenocarcinoma 1-lymphoma	11
Positive for malignancy	26 (57.8)	20-sqamous cell carcinoma 1-adenocarcinoma 4-poorly differentiated carcinoma 1-dysplasia high grade	14 (26.9)	12-adenocarcinoma 1-squamous cell carcinoma 1-high grade dysplasia	02 (15.4)	2-malignancy	42
Total	45		52		13		110

HPE=Histopathological examination

Table 2: Distribution of HPE findings of patients studied

HPE report	Esophagus (%)	Stomach (%)	Duodenum (%)	Total number of patients
Total nonneoplastic	11	35	08	54
Inflammatory lesion	04 (36.3)	32 (91.4)	07 (87.5)	43
Barrett esophagus	01 (9.1)	NA	NA	1
Normal	06 (54.5)	03 (8.6)	01 (12.5)	10
Total neoplastic	34	17	05	56
Squamous cell carcinoma	21 (61.8)	01 (5.9)	0	22
Adenocarcinoma	01 (2.9)	13 (76.5)	03 (60)	17
Poorly differentiated carcinoma	09 (26.5)	02 (11.8)	0	11
Lymphoma	0	0	01 (20)	1
Dysplasia	03 (8.8)	01 (5.9)	01 (20)	05
Total	45	52	13	110

HPE=Histopathological examination, NA=Not available

Table 3: Analysis of IC versus HPE for malignancy-site wise

	True positive	False positive	False negative	True negative	Total
Duodenum	5	0	0	8	13
Esophagus	32	0	2	11	45
Stomach	15	1	2	34	52
Total	52	1	4	53	110

IC=Imprint cytology, HPE=Histopathological examination

Candida was noted in one case that showed poorly differentiated carcinoma.

For gastric lesions, the sensitivity, specificity, diagnostic accuracy of IC were lower than the results of Sharma *et al*.

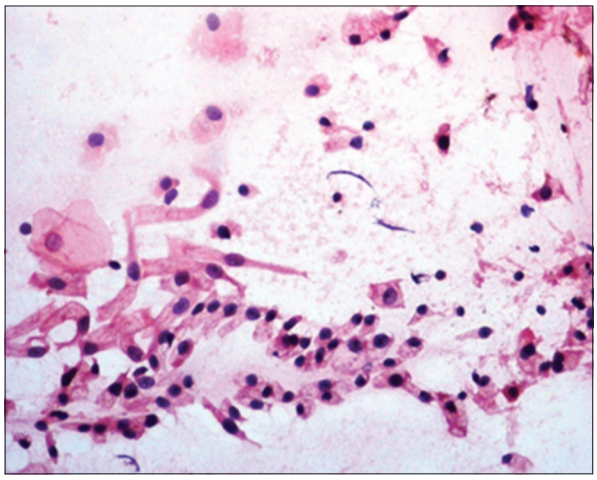

Figure 1: Barrett esophagus: Imprint smear showing benign columnar cells with admixed squamous cells (H and E ×200)

and Mysorekar *et al*.[2,3] The lower sensitivity was due to one unsatisfactory case and two false negative cases. In one case, imprint slides showed only necrosis, whereas fragments of tissue with carcinoma were seen on HPE. There was one false positive case due to regenerative atypia with cells showing nucleomegaly and prominent nucleoli, which was mistaken for malignancy.

Fourteen cases were reported as positive for malignancy, which showed tumor cells in clusters and acinar pattern with individual cells being columnar with mucin filled cytoplasm, vesicular nuclei, and prominent nucleoli. Signet ring cells were seen in one case [Figure 2].

Helicobacter pylori is associated with 2.9 fold increased risk of malignancy. In the present study, 10 cases (15.4%) out of

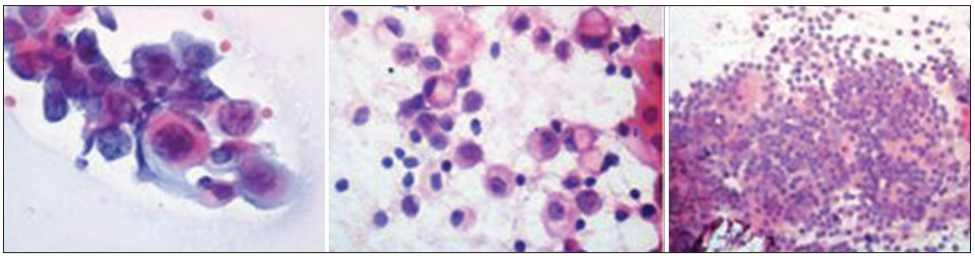

Figure 2: Squamous cell carcinoma: Imprint smear showing pleomorphic squamoid cells in clusters with orangeophilia of cytoplasm (Pap, ×400) (left). Signet ring cell adenocarcinoma: Imprint smear showing signet ring cells (H and E, ×400) (middle). Lymphoma duodenum: Imprint smear showing monomorphic dyscohesive cells with high N:C ratio obscuring columnar cells (H and E, ×400) (right)

Table 4: Analysis of IC versus HPE for *H. pylori* incidence-site wise

	True positive	False positive	False negative	True negative	Total
Duodenum	1	0	0	12	13
Stomach	7	0	2	43	52
Total	8	0	2	55	67

IC=Imprint cytology, HPE=Histopathological examination, *H. pylori=Helicobacter pylori*

65 cases including 52 gastric and 13 duodenal biopsies were positive for *H. pylori*. This was higher than 8% recorded by Kaur *et al*. in Malay population.[10] It was best demonstrated on air dried MGG stain.[11] *H. pylori* was identified by its spiral or S-shaped morphology. The presence of neutrophils, lymphoid aggregates and plasma cells helped in the diagnosis[12] [Figure 3].

Overall sensitivity of 80%, specificity 100%, and diagnostic accuracy 96.92%. of IC for *H. pylori* was recorded. There were two false negative cases probably due to low-density and patchy bacterial load. Kaur *et al*. recorded similar findings.[10]

Senturk *et al*. reported higher sensitivity for imprint smears than the brush.[13] Misra *et al*. recorded higher sensitivity and specificity of imprint smears combined with HPE compared to rapid urease test.[4]

For duodenal lesions, sensitivity, specificity and diagnostic accuracy of IC for duodenal neoplastic lesions were 100%, which was higher than that reported by Mysorekar *et al*.[3] This could be due to fewer cases in the present study. Among neoplastic lesions there were four cases of adenocarcinoma and one case of lymphoproliferative disorder, imprint smears of which showed monomorphic population of lymphoid cells. HPE showed lymphoepithelial lesions [Figure 2].

Chang *et al.* showed that the presence of a pathologist in the endoscopy suite to perform immediate assessment resulted in an adequate specimen in 100% of cases, as compared with only 71% when a pathologist was not present.[14] Further, imprinting did not damage the tissue for biopsy in any way.[4,15] Combined sensitivity of IC and histopathology is higher than imprint alone.[2] Assessment of depth of invasion and typing of tumors is done on histopathology.

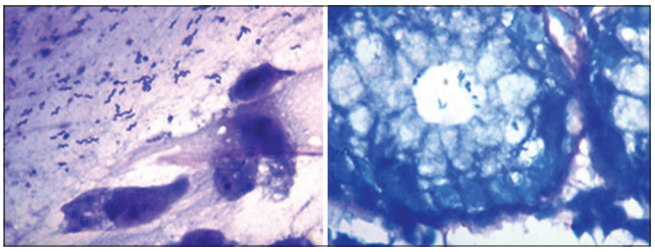

Figure 3: *Helicobacter pylori* gastritis: Imprint smear showing benign columnar cells with *H. pylori* organism (purple) in the background. (May-Grünwald-Giemsa [MGG], ×1000) (left). Tissue section of the above case showing *H. pylori* organism in the lumen of gastric glands. (MGG, ×1000) (right)

Conclusion

Imprint cytology is a valuable diagnostic aid for upper GI malignancies and deserves to be practiced more widely to determine adequacy of biopsy and to provisionally report as positive or negative for malignancy in a short period of time with minimum additional effort. This will enable early planning of further course of action by the clinician and help the patient by avoiding repeated procedures that may be required in case of inadequate biopsies.

References

1. Conrad R, ShobhaCastelino-Prabhu, Cobb C, Raza A. Role of cytopathology in the diagnosis and management of gastrointestinal tract cancers. J Gastrointest Oncol 2012;3:285-98.
2. Sharma P, Misra V, Singh PA, Misra SP, Gupta SC. A correlative study of histology and imprint cytology in the diagnosis of gastrointestinal tract malignancies. Indian J Pathol Microbiol 1997;40:139-46.
3. Mysorekar VV, Dandekar CP, Satyaprakash BS, Sarkar A. Role of imprint cytology in the diagnosis of gastrointestinal tract malignancies. Indian J Pathol Microbiol 2003;46:37-43.
4. Misra SP, Dwivedi M, Misra V, Gupta SC. Imprint cytology – A cheap, rapid and effective method for diagnosing *Helicobacter pylori*. Postgrad Med J 1993;69:291-5.
5. Geramizadeh B, Shafiee A, Saberfirruzi M, Kumar PK, Shaheem A. Brush cytology of gastric malignancies. Acta Cytol 2002;46:693-6.
6. Hughes JH, Leigh CJ, Raab SS, Hook SY, Cohen MB, Suhrland MJ.

Cytologic criteria for the brush diagnosis of gastric adenocarcinoma. Cancer 1998;84:289-94.
7. Young JA, Hughes HE. Three year trial of endoscopic cytology of the stomach and duodenum. Gut 1980;21:241-6.
8. Rosai J. Rosai and Ackerman's Surgical Pathology. 10th ed. Noida: Elsevier; 2011.
9. Yantiss RK. Diagnostic challenges in the pathologic evaluation of Barrett esophagus. Arch Pathol Lab Med 2010;134:1589-600.
10. Kaur G, Madhavan M, Basri AH, Sain AH, Hussain MS, Yatiban MK, *et al*. Rapid diagnosis of *Helicobacter pylori* infection in gastric imprint smear. Med J Malaysia 2002;57:67.
11. Rotimi O, Cairns A, Gray S, Moayyedi P, Dixon MF. Histological identification of *Helicobacter pylori*: Comparison of staining methods. J Clin Pathol 2000;53:756-9.
12. Xu XQ, Wang ZH, Liao JX, Chen XY, Liu WZ, Xiao SD, *et al*. Predictive value of neutrophil infiltration as a marker of *Helicobacter pylori* infection. World J Gastroenterol 2012;18:5101-5.
13. Sentürk O, Cantürk Z, Erçin C, Cantürk NZ, Celebi A, Hulagu S, *et al*. Comparison of five detection methods for *Helicobacter pylori*. Acta Cytol 2000;44:1010-4.
14. Chang KJ, Katz KD, Durbin TE, Erickson RA, Butler JA, Lin F, *et al*. Endoscopic ultrasound-guided fine-needle aspiration. Gastrointest Endosc 1994;40:694-9.
15. Stromar IK, Jakic-Razumovic J, Knezevic-Obad A, Knezevic J. Comparison of cytological and histological analysis of gastric mucosa biopsy in diagnosing malignant diseases. Lijec Vjesn 2004;126:287-90.

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