International Journal of Current Research and Review
DOI: http://dx.doi.org/10.31782/IJCRR.2021.13532

Scopus

Original Research

IJCRR
Section: Healthcare
ISI Impact Factor (2019-20): 1.628
IC Value (2019): 90.81
SJIF (2020) = 7.893

Diagnostic Role & Accuracy of Intra-Operative Frozen Section in The Surgeries for Gastric and Gastro-Esophageal Malignancies

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ABSTRACT

Introduction: Frozen section (FS) is an important and rapid intra-operative tool to assess primary pathology/ malignancy, diagnose metastatic malignancy and to ensure negative resection margins during the respective surgeries for gastric and gastro-oesophageal malignancies, which can subsequently impact patient prognosis and management.

Objective: To assesses the accuracy, sensitivity and specificity of intraoperative FS consultation in surgeries for gastric and gastro-oesophageal malignancies, in correlation with the final histopathology.

Methods: Over a period of 2 and ½ years, the results of gastric cancer patients who had undergone intraoperative FS consultation, were prospectively analyzed in comparison with the corresponding histopathology. The overall accuracy, sensitivity and specificity were calculated for all the specimen, as well as for margins. The discordant cases were reviewed to analyze the cause of the error.

Results: In total, 67 specimens were obtained from the 45 patients undergoing total and partial gastric resection surgery. The accuracy, sensitivity, and specificity of frozen-section assessments were 94.02%, 96% and 92.8% respectively for all the specimen. There were 4 discordant cases. The accuracy, sensitivity and specificity for margin assessment were 100%, 100% and 100% respectively.

Conclusion: Intraoperative FS examination is a highly accurate method which can be utilized for achieving rapid intra-operative diagnosis and negative margins, which is very essential for complete tumour extirpation in gastric and gastro-oesophagal malignancies.

Key Words: Frozen section, Histopathology, Discordance, Error

INTRODUCTION

Gastric cancer is the fourth most common cancer and the second leading cause of cancer death worldwidewith the only possible curative treatment being complete resection with negative surgical margins along with lymph node clearance.[1,2] Tumour cells in gastric cancer can spread intra-murally beyond the lesion, which is not macroscopically visible or palpable intra-operatively. Hence the resection margins must be far from the tumour edge to avoid the involvement of the margins by the malignancy. The short distance between tumour mass and resection margins also increases the possibility of local recurrence hence it is important to have a wide tumour free clearance margin with complete tumour extirpation.[3] Intra-operative consultation (IOC) by frozen section (FS) is frequently performed for assessment of surgical margins during surgeries for resection of gastro-oesophageal and gastric carcinoma to achieve tumour-free resection margins. The main purpose of the frozen section is to provide a rapid diagnosis to guide intra or perioperative patient management. Other than evaluation of margins, indications of the frozen section are the identification of tissue and detection of primary pathology/ lesion, identification metastasis in lymph node/ detection of metastatic malignancy in a suspicious nodule identified during intra-operative inspection/palpation and confirma-

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ISSN: 2231-2196 (Print) **ISSN:** 0975-5241 (Online)

Received: 16.10.2020 **Revised:** 24.11.2020 **Accepted:** 28.12.2020 **Published:** 03.03.2021

tion of the presence of representative samples for paraffin section diagnosis.

The purpose of this study is to analyze the accuracy of IOC by FS during surgeries of gastric and gastro-oesophageal malignancies and to review the causes of error.

MATERIALS AND METHODS

The present study was a prospective analysis is done in the department of pathology of a tertiary care hospital throughout 2 and ½ years during which all the intra-operative FS performed for patients undergoing resection surgery for gastro-oesophageal and gastric malignancy were analyzed. Fresh intra-operative tissue samples were included from general surgery and other surgical super-specialities like on-surgery and gastro-surgery. Patients undergoing gastric surgeries for non-neoplastic conditions were excluded from the study. Samples from operation theatre were transported immediately within a gauge piece soaked in normal saline to the laboratory. The request slip was verified for patient identification, clinic-radiological details and previous biopsy report if any. Tissue was examined and gross findings noted following which embedding, freezing and sectioning was done. Wet tissue was blotted with a paper towel to reduce freezing artefacts. Blocks were sectioned on LEICA CM1860 cryostat. The slides were stained with haematoxylin and eosin (H and E) stain and studied. In cases of positive margins, results were immediately informed to the surgeon over the phone and revised margins were sent till the final negative margin was achieved. The turn around time was noted per specimen. Tissue remainder were then fixed in 10% formalin solution and sent for histopathological examination after making paraffin embed sections and H and E Stain. IOC was done with 3 indications, i.e., 1. Margin assessment 2.Diagnosis of primary pathology/lesion 3.Evaluation of metastasis in lymph node/ suspicious nodule. Other than evaluation of margins during total/partial gastrectomy for gastric malignancy, diagnostic FS was also performed if the malignant diagnosis was suspected on clinical grounds, but biopsy results were negative. Such negative results included gastric ulcers macroscopically and clinically suggestive of malignancy, perforation in suspected malignancies requiring emergency intervention, or high-grade dysplasia/ intramucosal carcinoma.

The IOC results were compared with reports on their respective formalin-fixed, paraffin-embedded sections. The accuracy rate, sensitivity and specificity of the FS report for gastric surgeries were determined, comparing with the gold standard histopathology. Any discrepancy was noted and all cases with discordant diagnoses were reviewed for the cause of the error. The 'reasons for discordance' were categorized into three categories, i.e. 1-Interpretative errors, 2-sampling errors and 3- technical errors, after re-examination of all the slides with discordant diagnoses. Sampling errors included both gross and microscopic error. Gross sampling error occurred when the diagnostic material is not present in the FS sample, but tissue sent for histopathology other than frozen section, contained the diagnostic material or vice versa. Microscopic sampling error occurred when the diagnostic tissue was not seen on the frozen section itself (i.e. the block was not sectioned deep enough during intra-operative consultation) but subsequent deeper sections on the paraffin block of the same tissue showed the presence of diagnostic material. The reverse is also true, sometimes frozen section contains the pathologic lesion that was exhausted during intra-operative consultation hence not identified in the tissue submitted for permanent section. Surgical sampling error - Tissue not given from the representative site of the lesion. Technical errors – includes; suboptimal quality of the frozen section slide like tissue folding, improper freezing and staining.

Types of error in correlation- As described by the ADASP, the types of error in co-relation leading to a change in the final report were categorized under the following headings: 1. Change in the category, (i.e., from benign to malignant or vice versa) leading to false-positive or false-negative; 2.Change within the same category (e.g., the histological variant of malignancy); 3.Change in the status of the resection margin (i.e., false-positive or false-negative for malignancy); and 4.Change in lymph node status (i.e., false-positive or false-negative for malignancy).[4]

The study design was approved by the Research Ethics Committee of IMS and SUM Hospital, Bhubaneswar.

RESULTS

Over 2 and 1/2 years, intra-operative FS consultation was done in 45 cases of gastric surgery, which yielded 67 FS specimen for evaluation. Most of the patients were male (80%) and the mean age of presentation was 48.68. Of these, 24 cases (24 specimens) were sent with an indication of establishment/confirmation of primary pathology/malignancy, 12 cases (35 specimens) for assessment of margin status and 8 cases (8 specimens) were sent for assessment of metastatic malignancy in lymph node/suspicious nodule. 24 FS specimen were sent for establishment/ confirmation of malignancy, 12 of which presented clinically with gastric ulcers highly suspicious of malignancy, but negative for malignancy on biopsy, 4 cases presented with perforation, 5 cases with high-grade dysplasia and 3 cases with carcinoma-in-situ on biopsy. Of these 24, 14 were given the diagnosis of positive for malignancy in the final histopathology. Adenocarcinoma was the most common malignant diagnosis (13), of which 4 cases showed signet ring cell morphology and rest 9 were intestinal-type adenocarcinoma. Of the 8 cases sent for as-

sessment of metastatic malignancy, 3 were from peri-gastric lymph nodes, 3 were suspicious omental nodules, 1 omental lymph node and 1 from liver deposits, all of which showed the presence of metastatic adenocarcinomatous deposits on FS, which was later confirmed on histopathology of the remainder tissue (Table 1).

There were total 4 discordant diagnoses of which 2 were false positive (FP) and 2 were false negative (FN). Reason for discordance was misinterpretation in 2 cases sent for diagnostic frozen section and sampling error in 2 cases for margin evaluation. On review of the cases with interpretative error, we saw a case of Gastro-esophageal junction Carcinoid tumour was diagnosed as false negative on FS due to misinterpretation of tumour cells as normal lymphocytes of chronic inflammation. (Figure 1A) Subsequent paraffin sections showed a monotonous population of small round cells arranged in solid, insular and glandular pattern with finely granular cytoplasm, small nucleoli, salt and pepper chromatin.(Figure 1B, 1C) On subsequent IHC positivity of NSE in tumor cells, final histopathology impression of Carcinoid tumour was given. (Figure1D) In another discordant case due to interpretative error, review of a Gastric antral growth diagnosed as Adenocarcinoma on frozen section, permanent sections showed a diffuse infiltrate comprised of atypical lymphoid cells, and a final histopathological diagnosis of Non- Hodgkin's Lymphoma, B-cell type was confirmed after immunohistochemical positivity with LCA and CD20. The misinterpretation was due to freezing artifacts imparting the neoplastic lymphoid cells a fragile neoplastic epithelial morphology leading to a change in category. In both the cases with false-positive results, the reason for discordance was due to microscopic sampling error wherein the diagnostic material was exhausted during the intra-operative consultation, hence deeper sections of the block sent for histopathology did not show the tumour. Amongst the concordant cases, there was a case of gastric antral growth reported as adenocarcinoma on FS (Figure 2A). Subsequent paraffin sections showed neoplastic cells with moderate to marked nuclear pleomorphism arranged diffusely giving a concordant diagnosis of adenocarcinoma of the stomach (Figure 2B). IOC was done during a gastrectomy surgery for carcinoma stomach, wherein an omental lymph node was sent to diagnose for the presence of metastatic deposits. FS showed scattered neoplastic cells amidst lymphoid cells exhibiting moderate nuclear pleomorphism. An FS diagnosis of positive for malignancy was given (Figure 2C, 2D) which was subsequently confirm to be metastatic adenocarcinomatous deposits on histopathology (Figure 2E, 2F)

Of the 67 specimens submitted, FS diagnosis was concordant with histopathology in 63 specimens and discordant in 4 specimens. In comparison with the permanent sections (PS), there were 24 true positives (TP), 39 true negatives (TN), 2 false positives (FP) and 2 false negatives (FN) diagnoses. (Table 1) The overall accuracy of 94.02% (95% CI: 85.4, 98.3). The sensitivity and specificity of intraoperative FS in gastric surgeries were found to be 96% (95% CI: 79.6, 99.9) and 92.8% (95% CI: 80.5, 98.5) respectively. The positive predictive value and negative predictive value for FS in detecting stomach pathologies were found to be 88.9% (95% CI: 72.8, 95.9) and 97.5% (85, 99.6) respectively (Table 2). The positive likelihood ratio and negative likelihood ratio of FS were found to be 13.44 and 0.04 respectively. Hence the FS had a weak LR+ and LR- values for the specimen from Gastric surgeries.

In the total of 13 cases, IOC was done for margin assessment, which yielded 35 specimens for evaluation. Of these, 17 were gastric margins, 13 were oesophagal margins and 5 were small bowel margins. Of these 6 margins (17%) showed the presence of tumour tissue, which was reported as Margin positive for malignancy in the FS report. In all the cases with positive margins, revised margins were sent till the final negative margin was achieved. The overall accuracy of FS for margin assessment in gastric surgeries was 100%. The sensitivity and specificity of FS in margin assessment was found to be 100% (95% CI: 54.1, 100) and 100% (95% CI: 88.1, 100) respectively. Receiver operating curve (ROC) area was found to be 1 (95% CI: 0.67, 0.75) thus justifying that FS can be used to detect margins against the gold standard for samples collected for margin assessment during Gastric surgeries. The positive predictive value and negative predictive value for FS were the same as sensitivity and specificity.

DISCUSSION

The purpose of FS on surgical margins, unknown lesions and lymph nodes/nodules is to assure complete tumour extirpation. Our study found that the overall accuracy of FS during gastric surgeries was 94.02%, sensitivity was and specificity was which is comparable with previously reported data in the literature. The overall discordance rate in this study was 5.98% (2.99% FN and 2.99% FP results).

McAuliffe et al reported overall specificity of 99.8%, the sensitivity of 77.0%, the positive predictive value of 96.3%, the negative predictive value of 98.2% for the IOC on surgical margins. They reported an overall diagnostic accuracy of 98.1% for all IOC of gastric and gastro-oesophageal surgeries, which is comparable to our study. They also found that signet ring cell and diffuse-type final diagnoses had higher rates of FN results.[5] Spicer et al. reported their experience with IOC during margin assessment of gastro-oesophageal and gastric adenocarcinoma surgeries and shed light the diagnostic difficulty posed in signet ring cell disease.[13-15] Of the 6 cases with FN IOC results, 5 were signet ring disease.[6] Challenges in the FS examination of gastrointestinal

poorly cohesive and signet ring cell carcinomas have also been discussed by Zhu et al.[7] In our study, the challenges were sampling errors leading to false-positive diagnoses and misinterpretation due freezing artifacts leading to bloated cell morphology. Squires et al noted 13% of positive proximal margins, Celli et al. reported 21% positive margins and Berlth et al. reported 1% margin positivity, as compared to 17% positive margins seen in our study.[8,16,17] The CAP has established benchmarks for anatomical pathologic error based on large studies.[9-12] that evaluated the discordance rate of FS consultation in comparison with final pathologic diagnoses. In IOCs performed for margin assessment, the discordance rate was 0.00%, which was within the CAP benchmarks (discordance rate of approximately 2.0% for neoplastic cases, based on the studies).[10-12] Accuracy of FS in margin assessment in our study was 100%, which is in line with the findings of other authors (Table 2).

CONCLUSION

Tumour infiltration at resection lines (positive resection margins) can have an adverse prognostic factor with increased chances of tumour recurrence. Hence negative resection margins and complete tumour extirpation are of utmost importance in the curative resection for gastric malignancies. FS, though it provides rapid intra-operative diagnosis, which can help the surgeon in rapid intra-operative decision-making, this technique has its limitations. As has been the experience at our institute, despite the challenges in sampling and difficulties in interpretations due to technical errors, the results of FS for gastro-oesophageal and gastric malignancies are accurate, especially for margin assessment and comparable to the results of other studies in the literature. With effective communication and correlation of relevant clinic-radiological and other investigational data between the operating surgeon and the pathologist, the error rates can be minimized. The results of this study may shed light on the importance of FS at the time of resection of the gastro-oesophagal junction and gastric malignancies, which acts as a guiding hand for the operating surgeon to decide the appropriate course of management for the patient intra- and peri-operatively.

Conflict of interest – Nil.

Financial support - Nil.

Authors' Contribution:

MR-manuscript writing, editing, literature search, drafting and data acquisition.

PM- concept, design, manuscript editing.

SA- clinical studies, data acquisition.

ACKNOWLEDGMENT

The author acknowledges the help received from Professor and Head, Department of for her guidance. I am also thankful to my Guide for her kind co-operation and meticulous supervision of the work. I would like to give my special thanks to all the technicians of Histopathology and cytology Section, for helping me while conducting the present study. The author is also grateful to authors/editors/publishers of all those articles, journals and books from where the literature for this article has been reviewed, discussed and cited.

ABBREVIATIONS

FS- Frozen section

PS- Permanent section

IOC- Intra-operative consultation

H and E- Hematoxylin and eosin.

FP- False positive

TP- True positive

FN False negative

TN- True negative

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Table 1: Correlation of intra-operative FS diagnosis done in Gastric surgeries with the final HP diagnosis

Indication	No. of cases	No. of specimen	FS Diagnosis		PS Diagnosis		TP	TN	FP	FN
			Positive	Negative	Positive	Negative				
Diagnostic	24	24	15	9	14	10	13	7	2	2
Margins	13	35	6	29	6	29	6	29	0	0
Metastasis	8	8	5	3	5	3	5	3	0	0

Table 2: Different studies showing the accuracy of Frozen Section in gastric surgeries as compared to Histopathology

Name of the study	Site of lesion	No. of specimen	Accuracy/ Concordance rate	Sensitivity	Specificity	Reference, Remarks
Spicer et al, 2014	Gastric and esophageal, margins	122	93%	67%	100%	6
Chatelein et al, 2012, France	GIT	800	96.6%			13
Shen et al, 2006	Gastric cardia, margins	66	97%	77.8%	100%	14
Nakanishi et al, 2019	Gastric, margins	1241	99.4 %	99.5%	97.8%	15
Berlth et al, 2020	Gastric, margns	1484	99.4%			17
Present study	Gastric	75	94.03%	96%	92.8%	

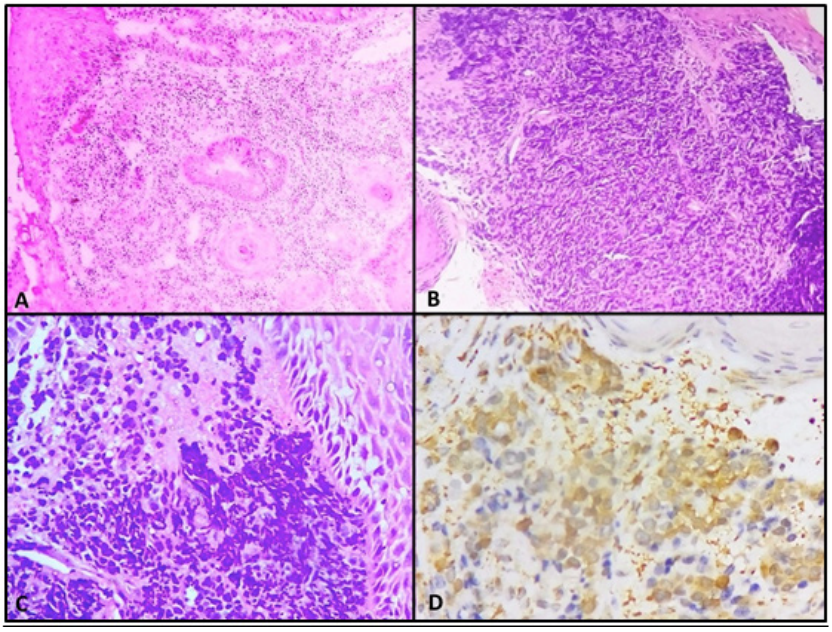

Figure 1: A. FS from GEJ growth reported as negative for Malignancy in Frozen section (FS, H and E, 100x). B. Subsequent PS of the same case showing the presence of Carcinoid tumour (PS, H and E, 100x). C. Other areas of PS of the same case showing the presence of Carcinoid tumour (PS, H and E, 400x). D. Final HP Impression- Carcinoid tumour, GEJ, confirmed by NSE Positivity (PS, NSE, 400x).

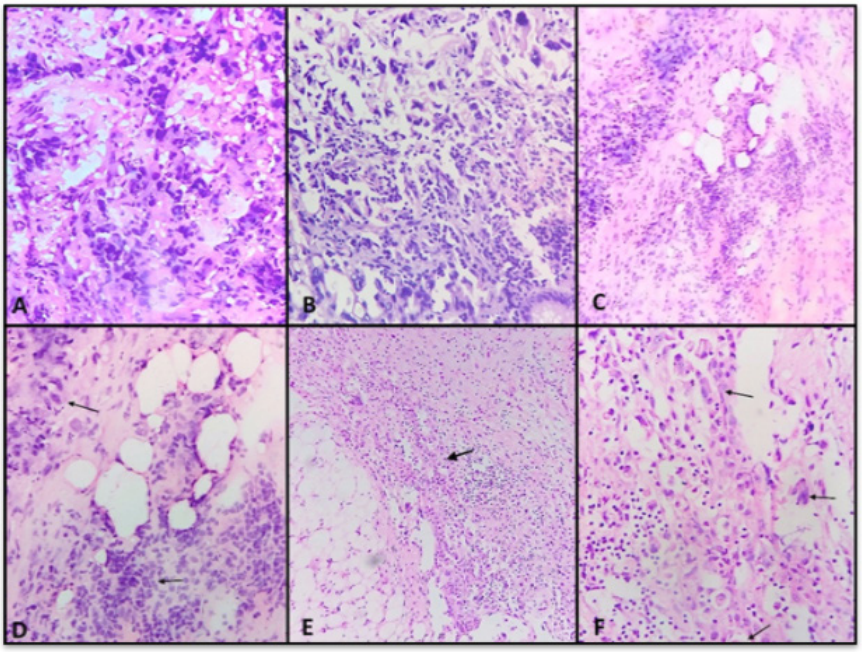

Figure 2: A. FS from a gastric antral growth Reported as Adenocarcinoma, Stomach (FS, H and E, x400). B. Subsequent PS given final histopathological diagnosis of Adenocarcinoma, Stomach (PS, H and E, x400). C. FS of an Omental Lymph node,given diagnosis of Metastatic adenocarcinomatous deposits on FS(FS, H and E, x100). D. Higher power view of the same field showing presence of tumor cells scattered singly and in small clusters (arrow) amidst lymphoid cells (FS, H and E, x400) E. PS of the same node showing the presence of tumour tissue(arrow) amidst the lymphoid cells(PS, H and E, x100). F. Higher power view of the same field showing the presence of scattered tumour cells (arrow) rendering a final histopathological diagnosis of Metastatic Adenocarcinomatous deposits, Omental Lymph node. (PS, H and E, x400).