Original Article

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Intraoperative Frozen Section Analysis and an Audit of its Diagnostic Accuracy: A Cross-sectional Study from Maharashtra, India

Pathology Section

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ABSTRACT

Introduction: Frozen Section (FS) is a diagnostic technique performed intraoperatively to obtain relevant information about the primary diagnosis, margin status, or sentinel lymph nodes, which helps guide the course of surgery. With the prevalent use of FS, it is imperative to recognise and scrutinise its diagnostic pitfalls and make efforts for rectification.

Aim: To compare diagnostic results obtained on FS with final paraffin-embedded histopathology and calculate the Diagnostic Accuracy (DA) of FS.

Materials and Methods: This cross-sectional study was conducted in the Department of Pathology at a tertiary care hospital, DVVPFs Medical College in Ahmednagar district, Maharashtra, India over a period of two years from January 1, 2020, to December 31, 2021. The diagnosis provided on specimens received for intraoperative FS was compared with the final histopathological diagnosis, considered as the gold standard for the same specimen. The results were categorised as concordant, discordant, and deferred cases. The diagnosis on FS and the final histopathological diagnosis, along with relevant clinical data, were entered into an excel sheet. Further, the DA of FS was calculated. All the discordant cases were analysed. Statistical analysis utilised simple percentage calculations from the excel sheet.

Results: A total of 130 cases were analysed using FS, with the most common indication being sentinel lymph node analysis to detect metastatic deposits in 72 (55.4%) cases. Out of the total 130 cases, 119 (91.5%) were concordant, 10 (7.7%) were discordant, and 1 (0.8%) was deferred due to an insufficient specimen. Therefore, the overall DA rate was found to be 91.5% with an error rate of 8.5%. Upon analysis of the 10 discordant cases, the cause of inaccuracy was technical error in 7 (70%) cases and interpretation error in 3 (30%) cases.

Conclusion: The DA obtained in the present study was somewhat lower than expected due to technical errors during FS sectioning, leading to artifacts, especially during lymph node processing. Analysis of the discordant cases unveiled this deficit. Therefore, such assessment studies should be performed periodically as they assist in highlighting the shortfalls and provide a plan to boost DA.

Keywords: Diagnostic technique, Discordant, Postoperative histopathology

INTRODUCTION

Intraoperative FS is a technique wherein tissue specimens obtained during an operation are processed and analysed by the pathologist for expedited consultations. It combines surgical procedures with pathological expertise to obtain the most advantageous results for patient management [1,2]. This technique was introduced and perfected by Dr. Louis B. Wilson in 1905, followed by several advancements thereafter with the advent of the cryomicrotome, also known as the cryostat [3,4]. FS requires absolute coordination between the surgeon, residents, and the pathologists, with the starting point being the obtaining of the tissue specimen from the patient in the operation theatre. This unfixed specimen is transported immediately to the Pathology Department, followed by the preparation and examination of slides. The diagnosis is then conveyed to the surgeon-in-charge [3].

Above all, it is imperative for the pathologists and operating surgeons to be conscious of the indications and limitations of this procedure. Routinely, FS is employed to detect the presence of a tumour or metastasis in a suspected tissue, identify the primary diagnosis or the benign/malignant nature of a lesion, determine the resected margin accuracy, and assess tissue adequacy and viability [4-6]. Fresh tissue samples can also be obtained for ancillary techniques like electron microscopy and molecular studies [4,5]. Despite the exceptional usefulness of FS, pathologists can face obstacles in reaching the final diagnosis owing to errors in sampling the tissue, technical hindrances, and during the interpretation of the slides. The anatomical sites also lead to variability in DA [4,7].

Due to the well-approved usage of FS, it is essential to conduct a periodic evaluation to unearth diagnostic pitfalls to reduce misinterpretations and improve diagnostic precision. Therefore, the aim of the present study was to compare the diagnostic results obtained during FS with the final diagnosis rendered on paraffin sections and to calculate the DA of FS. Also, the cases which were misdiagnosed were reviewed to detect diagnostic fallacies for future improvements.

MATERIALS AND METHODS

This cross-sectional study was conducted at the Department of Pathology, DVVPFs Medical College, Ahmednagar, Maharashtra, India, from 1st January 2020 to 31st December 2021 over a period of two years.

Inclusion and Exclusion criteria: All the intraoperative frozen specimens sent for histopathological analysis were included in the study. Since the analysis of every case sent for FS was to be performed, no exclusion criteria were kept.

Ethical clearance was obtained from the Institutional Ethics Committee (IEC no. ECR/787/Inst/MH 2015) on 8th August 2019.

Study Procedure

Technique of frozen sectioning: Before the reception of FS in the Department of Pathology, the surgeon-in-charge verbally informed the pathology consultant of the same. All the samples were sent unfixed in normal saline, accompanied by a completed requisition form containing the age and sex of the patient, type of specimen

sent, and the possible clinical diagnosis. The time of specimen reception was noted down.

The samples were examined grossly, and adequate representative sections were taken, which were transferred onto chucks. A tissue freezing media was used to immediately freeze the tissue bits, following which sectioning was performed at -18°C to -24°C using a cryostat (Leica CM1520) [Table/Fig-1], where a minimum of two sections, 5 µm thick, were taken and shifted onto glass slides. These sections were stained using a rapid Haematoxylin and Eosin (H&E) procedure and reported independently by two pathologists. The final diagnosis was immediately conveyed to the surgeon-in-charge, and the reporting time was noted down. The Turnaround Time (TAT) was calculated from the specimen reception time and reporting time. The rest of the tissue specimen was fixed in 10% neutral-buffered formalin and processed routinely.

[Table/Fig-1]: An image of cryostat machine (Leica CM1520).

Diagnostic categories: A comparison was made between the diagnosis rendered on FS and final formalin-fixed tissue sections, and the results were categorised as concordant, discordant, and deferred categories. The cases with inadequate material and dubious histopathological findings with no definite opinion were classified as deferred cases. The DA of FS was calculated from the raw excel sheet data by simple calculation using the formula TP+TN/TP+TN+FP+FN X 100. In case of the discordant cases, the causes for discrepancy were recorded and scrutinised.

STATISTICAL ANALYSIS

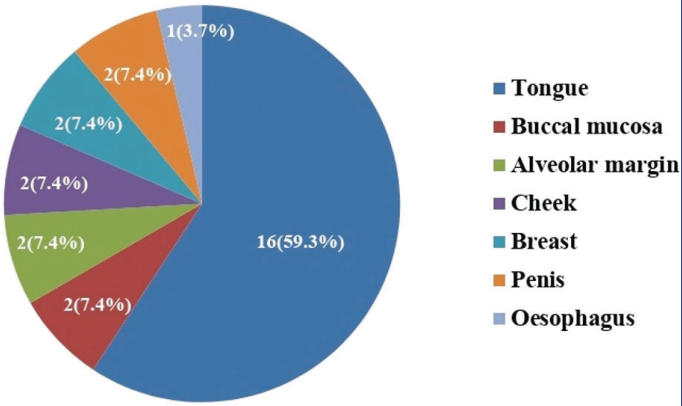
Relevant numerical and clinical data were tabulated in an excel sheet. Comparisons were made between diagnosis given on FS and the final histopathological diagnosis (gold standard). Statistical analysis utilised simple percentage calculations to yield overall and organ system-wise DA. Overall sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) were calculated. These parameters were also evaluated for the 72 cases of sentinel lymph nodes, as they alone comprised 55.4% of the cases in the present study.

RESULTS

A total of 130 intraoperative FS cases were included in the study [Table/Fig-2]. The patient age range varied from a minimum of 18 years (for intestinal polyp) to a maximum of 98 years (for sentinel lymph node analysis) with the mean age being 52 years. Female patients formed the majority with 93 (71.5%) specimens, while the specimens received from male patients numbered 37 (28.5%). The most common indication for FS in the present study was the detection of metastatic deposits in sentinel lymph nodes in 72 (55.4%) cases, followed by margin assessment for tumour cells from various sites in 27 (20.8%) cases [Table/Fig-3], and the establishment of the primary tumour diagnosis in 31 (23.8%) cases. Out of the 72 sentinel lymph nodes sent for intraoperative FS, the axillary group comprised the majority, with 54 (75%) cases, followed by the inguinal group with 14 (19%) cases and the cervical group with 4 (6%) cases. The axillary group was predominantly sampled to look for metastatic deposits due to invasive breast carcinoma.

Organ system	Anatomical site of specimen	No. of specimens	Percentage	Total no. of specimens
Sentinel lymph nodes	Axillary	54	41.5%	72 (55.4%)
	Inguinal	14	10.8%	
	Cervical	4	3.1%	
Surgical resection margin assessment from oral cavity	Tongue	16	12.4%	22 (16.9%)
	Buccal mucosa	2	1.5%	
	Alveolar margin	2	1.5%	
	Cheek	2	1.5%	
GIT	Colon and rectum	4	3.1%	10 (7.7%)
	Appendix	3	2.2%	
	Periampullary region	1	0.8%	
	Stomach	1	0.8%	
	Oesophagus (for resection margin assessment)	1	0.8%	
Female reproductive system	Ovary	10	7.7%	19 (14.6%)
	Breast (2 cases for margin assessment)	6	4.6%	
	Uterus	3	2.3%	
Male reproductive system	Penis (for resection margin assessment)	2	1.5%	3 (2.3%)
	Testes	1	0.8%	
Soft-tissues	-	2	1.5%	2 (1.5%)
Skin	Nasolabial fold	1	0.8%	1 (0.8%)
Endocrine	Parathyroid gland	1	0.8%	1 (0.8%)
Total	-	130	-	130 (100%)

[Table/Fig-2]: Site-wise distribution of all the specimens received for FS (n=130).



[Table/Fig-3]: Site-wise distribution of specimens received for margin status assessment (n=27).

Out of the total 130 cases received for FS, 119 (91.5%) cases were concordant, 10 (7.7%) cases were discordant, and 1 (0.8%) case had to be classified in the deferred category due to inadequate material leading to debatable findings on FS [Table/Fig-4]. Thus, the overall DA of FS in the present study was 91.5%. The overall sensitivity, specificity, PPV, and NPV were 96.5%, 87.5%, 86.2%, and 96.9%, respectively. The average TAT for FS reporting in the present study was 18 minutes. Among the 10 (7.7%) discordant cases, the basis

Site of specimen	No. of FS specimens (%)	Diagnosis type on HPE	No. of cases according to HPE diagnosis	Concordant cases	Discordant cases	Deferred cases	Overall DA
Sentinel lymph node	72 (55.4%)	For metastatic deposits	72	66	6	0	91.7%
Oral cavity	22 (16.9%)	Margins clear	17	17	0	0	100%
		Margins involved	5	5	0	0	
Ovary	10 (7.7%)	Benign	9	8	1	0	90%
		Borderline	1	1	0	0	
GIT	10 (7.7%)	Benign	6	5	0	1*	90%
		Malignant	3	3	0	0	
		Margins clear	1	1	0	0	
Breast	6 (4.6%)	Margins clear	1	1	0	0	83.3%
		Margins involved	1	1	0	0	
		Benign	2	1	1	0	
		Malignant	2	2	0	0	
Uterus	3 (2.3%)	Benign	3	1	2	0	33.3%
Soft-tissue	2 (1.5%)	Benign	1	1	0	0	100%
		Malignant	1	1	0	0	
Penis	2 (1.5%)	Margins clear	2	2	0	0	100%
Testes	1 (0.8%)	Benign	1	1	0	0	100%
Nasolabial fold	1 (0.8%)	Malignant	1	1	0	0	100%
Parathyroid gland	1 (0.8%)	Benign	1	1	0	0	100%
Total	130 (100%)	-	130	119	10	1	91.5%

[Table/Fig-4]: Distribution of FS cases according to site along with concordant and discordant cases and Diagnostic Accuracy (DA) (N=130).
HPE: Histopathological examination; *Deferred due to inadequate sample for FS

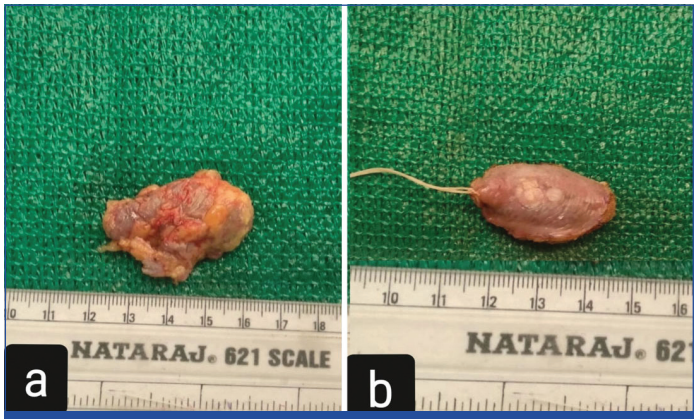
for discrepancy was due to technical error in 7 (70%) cases and interpretation error in 3 (30%) cases. The technical errors included sectioning error in 6 (85.7%) cases and sampling error in 1 (14.3%) case [Table/Fig-5].

Sample type	No. of cases	Frozen reported as	HPE reported as	Cause of error
Axillary lymph node	4	Positive for metastatic deposits	Negative	Technical error (sectioning error)
Axillary lymph node	1	Negative for metastatic deposits	Positive	Technical error (sectioning error)
Cervical lymph node	1	Positive for metastatic deposits	Negative	Technical error (sectioning error)
Ovary	1	Serous cystadenocarcinoma	Sertoli leydig cell tumour	Interpretation error
Breast	1	Infiltrating ductal carcinoma	Fibrocystic disease	Technical error (Tissue sampling)
Uterus	1	Low-grade Leiomyosarcoma	Cellular leiomyoma	Interpretation error
Uterus	1	Low-grade Leiomyosarcoma	Giant leiomyoma	Interpretation error

[Table/Fig-5]: List of discordant cases with possible reasons (n=10).

Out of the 72 sentinel lymph nodes sampled [Table/Fig-6a], 66 (91.7%) were concordant, whereas a discrepancy was noted in 6 (8.3%) cases. Among the six discordant cases, 5 (83.3%) belonged to the axillary group, while 1 (16.7%) was the cervical sentinel lymph node. The sensitivity, specificity, PPV, and NPV of FS in detecting sentinel lymph node metastasis was 96%, 89.4%, 82.7%, and 97.7%, respectively, in the present study.

The second most commonly sampled site was the oral cavity. Out of the total 130 specimens, 22 (16.9%) were sampled from the oral cavity for margin status assessment. Among these, 16 (72.7%) cases were glossectomy specimens [Table/Fig-6b], followed by 2 (9.1%) cases each from buccal mucosa, alveolar margin, and cheek. All 22 cases were concordant with the final histopathological findings, resulting in a 100% DA in Frozen Section (FS) analysis. Five (22.7%) cases out of the total 22 had positive resection margins in FS, leading to further revision surgery. Ovarian and Gastrointestinal Tract (GIT) specimens comprised 10 (7.7%) specimens each out of the total 130 specimens.

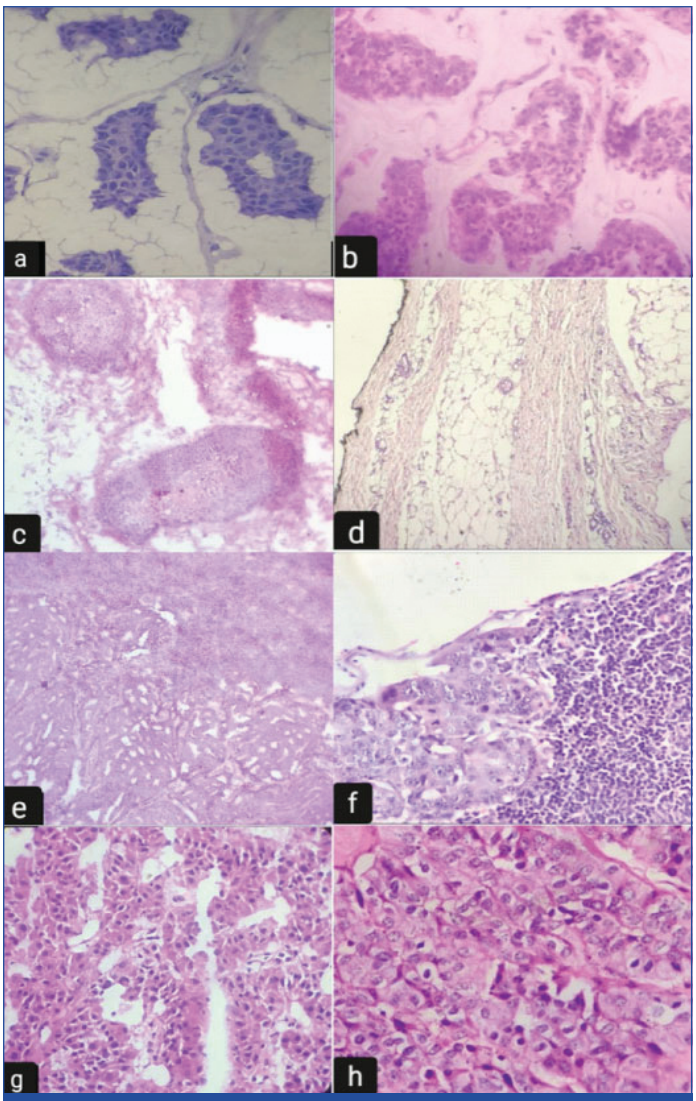


[Table/Fig-6]: a) Inguinal lymph node specimen in a case of carcinoma penis for FS; b) glossectomy specimen for margin evaluation on FS.

The final histopathological diagnosis of ovarian lesions included nine benign and one borderline malignant case, comprising benign cystic lesions, mature cystic teratomas, and surface epithelial tumours. The overall DA for ovarian lesions was 90%, with 1 (10%) case of sertoli leydig cell tumour wrongly diagnosed as serous cystadenocarcinoma, resulting in a DA of 89% for benign ovarian lesions. GIT specimens included specimens from the colon, rectum, appendix, stomach, periampullary region, and oesophagus, with diagnosis comprising mild colitis, polyps, mucocele of appendix, carcinomas, and negative findings for tumour cells in margin assessment of the oesophagus. One (10%) case from the rectum was deferred and later diagnosed as mild colitis on postoperative histopathology. The DA in GIT specimens was 90%.

A total of 6 (4.6%) specimens were received from breast tissue, two for margin assessment and the remaining four comprising fibrocystic disease, fibroadenoma, and invasive carcinoma. The DA was 83.3%, with fibrocystic disease wrongly diagnosed as invasive carcinoma on FS. An unsatisfactory DA of 33.3% was observed in uterine specimens, where cellular leiomyoma and giant leiomyoma were wrongly diagnosed as low-grade leiomyosarcoma on FS. The remaining 7 (5.4%) specimens received from soft tissue, penis, testes, nasolabial fold, and parathyroid gland were concordant with diagnosis being spindle cell sarcoma, negative for tumour cells in margin assessment,

orchitis, basal cell carcinoma, and parathyroid adenoma, respectively. A photomontage of four interesting concordant cases depicting histopathological features on FS and their corresponding paraffin-embedded sections has been included [Table/Fig-7a-h]. These cases emphasise the importance of appropriate grossing, sectioning, and careful microscopic examination of sections. Multiple sections had to be studied to correctly identify mucin in mucinous breast carcinoma, resection margin positive for tumour cells, and metastatic deposits in lymph nodes. Correct identification of parathyroid adenoma on FS reinforces the importance of communication between the pathologist and the surgeon-in-charge for the correlation of gross and microscopic features.



[Table/Fig-7]: Histomorphology on FS and corresponding HPE sections: (a,b) photomicrograph showing invasive mucinous breast carcinoma on FS and HPE respectively (H&E, x400); (c) photomicrograph showing circumferential margin positive for tumour cells in a breast lumpectomy specimen (rapid H&E, x400); (d) revised margins (inked) sent, do not show presence of tumour cells on HPE (H&E, x400); (e,f) presence of metastatic deposits in sentinel lymph node on FS and HPE respectively (H&E, x400); (g,h) Benign parathyroid adenoma showing tumour cells arranged in trabecular pattern and solid sheets with presence of nuclear pleomorphism and mitotic figures on FS and HPE, respectively (H&E, x400).

DISCUSSION

Intraoperative FS has gradually established itself as an advantageous practical tool in surgical pathology, primarily for crucial and life-altering decisions made on the operating table, including sentinel lymph node metastasis, surgical margin status, and primary diagnosis. For an FS report to be made available, the team in charge needs to function as a well-oiled machine with good communication and technical proficiency. Thus, it is crucial for every institution to maintain a system of checks and balances in the form of periodic audits.

The FS reporting, apart from being accurate, needs to be as prompt as possible. According to CAP, the standard Turnaround Time (TAT) for FS should be within 20 minutes [8]. In the present study, the average TAT was 18 minutes, which is comparable to the studies conducted by Devi J, Diwagar N, Sekhar G, and Vimal M [7-9]. The average TAT in these studies was 20, 15, and 15 minutes, respectively.

Most of the specimens received were from female patients (71.5%), similar to other studies by Maurya VP et al., (57%) and Diwagar N and Sekhar G (91%), which can be attributed to the requirement of FS analysis in axillary sentinel lymph node in breast cancer and obstetrics and gynaecology cases [6,8]. The most common indication for FS in the present study was sentinel lymph node assessment, accounting for 72 (55.4%) cases. However, the most common indication in studies conducted by Selvakumar AS et al., and Agarwal P et al., was margin assessment, at 52.2% and 34.8%, respectively [2,5]. Additionally, studies conducted by Maurya VP et al., and Diwagar N and Sekhar G reported primary tumour diagnosis as the commonest indication, at 66.5% and 95.6%, respectively [6,8].

The overall DA in the present study was 91.5%, which is slightly lower than in other prior studies in the literature, where the accuracy ranged from 94% to 98% [Table/Fig-8] [2,3,5-10]. Latest studies conducted on this subject were also compared with the present study, with Ayyagari S et al., Bharadwaj BS et al., and Chavda CI et al., displaying a DA of 98.5%, 95.5%, and 86.6%, respectively [11-13]. The first two studies [11,12] have comparable results with the previous studies; however, in the third study [13], below-average DA was observed, as their sample design included cases from the Central Nervous System (CNS) predominantly, which may prove to be a challenge during FS diagnosis.

In the present study, one possible reason for a discordance rate of 8.5% could be the inclusion of difficult-to-diagnose cases of uterine smooth muscle origin on FS. Both wrongly diagnosed

Authors	Place of study	Year of study	Study period (year)	Number of cases	Concordance rate	Discordance rate
Roy S et al., [3]	Pittsburgh (USA)	2013	9 months	327	97.6%	2.4%
Vimal M [9]	Puducherry	2015	1	140	97.8%	2.2%
Patil P et al., [10]	Wardha, (Maharashtra)	2015	2	100	96.9%	3.1%
Agarwal P et al., [5]	Lucknow (U.P)	2016	2	224	94.2%	5.8%
Selvakumar AS et al., [2]	Chennai (Tamil Nadu)	2018	5	518	98.6%	1.4%
Diwagar N and Sekhar G [8]	Chennai (Tamil Nadu)	2019	2	160	96.0%	4.0%
Maurya VP et al., [6]	Panchkula (Haryana)	2020	2	212	97.2%	2.8%
Devi J [7]	Guwahati (Assam)	2020	15 months	53	96.2%	3.8%
Ayyagari S et al., [11]	Hyderabad (Telangana)	2021	2	66	98.5%	1.5%
Bharadwaj BS et al., [12]	Guwahati (Assam)	2022	2	200	95.5%	4.5%
Chavda CI et al., [13]	Ahmedabad (Gujarat)	2022	27 months	201	86.6%	13.4%
Present study	Ahmednagar (Maharashtra)	2023	2	130	91.5%	8.5%

[Table/Fig-8]: Comparison of Diagnostic Accuracy (DA) with other studies [2,3,5-10].

cases of leiomyoma can be attributed to interpretation errors. The altered cell morphology, along with aberrant growth patterns of cellular leiomyoma and myxoid change in the case of giant leiomyoma, led to these two cases being erroneously diagnosed as low-grade leiomyosarcoma [14]. For the most part, diagnostic inaccuracies in FS can be explained by errors in technicality and interpretation [5]. Apart from the above-mentioned uterine lesions, a gross interpretation error was also observed in one case of an oophorectomy specimen, where a faulty diagnosis of serous cystadenocarcinoma was rendered on FS instead of the actual diagnosis of sertoli leydig cell tumour. The discrepancy was probably due to an inability to recognise a dual cell population on FS, along with focal papillary-like areas.

In the present study, all six discordant cases of sentinel lymph nodes can be attributed to technical errors during lymph node sectioning, leading to thicker and folded sections along with a chattering artifact. Sampling error was identified in one case of a breast lesion for the primary diagnosis, where benign fibrocystic disease was incorrectly labeled as invasive ductal carcinoma due to a few studied sections. The authors were unable to sample the cystic areas on FS; rather, the sections studied displayed a few clusters of atypical-looking cells, contributing to the diagnosis of invasive ductal carcinoma.

The overall DA for breast lesions in the present study was 83.3%, which is quite low compared to the DA of 98.5% obtained by Sheikh SA et al., [15]. The basis for this low DA is a consequence of very few cases of breast lesions examined in FS in the present study. Previous studies demonstrate a deferral rate of 0 to 6.1%, while a deferral rate of 1 (0.8%) case was observed in the present study due to insufficient sample leading to obscure histopathological findings [2].

Analysis of FS has provided valuable insights and assisted them in identifying deficient areas with scope for improvement. A deeper understanding of the diagnostic pitfalls of FS is required to minimise diagnostic inaccuracies. It is imperative to correlate the clinical parameters, gross findings, and microscopic findings of the specimen before providing an FS diagnosis. Adequate sampling from diverse regions to correctly identify the microscopic spectrum in the case of large specimens should be done. Requisite technical training should be provided to laboratory personnel to avoid errors during tissue processing. Nevertheless, it is always better to defer a doubtful case with unclear microscopic findings rather than providing a wrong diagnosis on FS.

Limitation(s)

The limitation of the study was the lack of diverse specimen and sample types for the primary diagnosis on FS, leading to a less comprehensive study of FS accuracy.

CONCLUSION(S)

Intraoperative FS has long been integrated as a prompt diagnostic technique in determining the course of surgical procedures. A thorough examination of the gross specimen with representative sampling for microscopy, suitable sectioning, and technical awareness while using the cryomicrotome, along with finer interpretation of microscopic slides, will go a long way toward improving the DA of intraoperative FS.

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