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Advancements in intra-operative histology consultation through frozen section techniques

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Abstract
Background: Frozen section (FS) technique was first introduced by the William H. Welch, from John Hopkins Hospital in 1891. By the early and mid-1920s, the technique became popular and was used for intra-operative consultation practice. The preparation of frozen section was made easier in the 1950s and 1960s by the development of the modern cryostat, a cabinet cooled to - 20 to - 30 degree Celsius and enclosing a microtome blade. Frozen section is a multistep process involving surgical resection, preparation of slides and their microscopic examination, communicating frozen section diagnosis to surgeon and processing the remaining tissue for further work up. The main purpose of frozen section is to provide rapid diagnosis to guide intra or peri-operative patient management.
Objective
- Identification of tissue and unknown pathological processes.
- Evaluation of margins.
- Identification of lymph node metastasis.
- Co-relation of frozen section diagnosis with routine histo-pathological diagnosis.

Materials and Methods: The present study was carried out on 50 patients. The gross specimens were cut into thin slices and examined. Sections were taken from abnormal, suspected areas, or from firm lesions and placed in a mounting medium (OCT).
Results: Overall Concordance rate of 94% was obtained for Frozen section & histopathological diagnosis with 86.7% accuracy in ovary cases, 83.3% accuracy in breast cases and 100% accuracy in testis, GIT, thyroid, endometrium, salivary gland, retroperitoneal region, brain tissue and margins evaluations.
Conclusion: The frozen section diagnosis is very useful for intra-operative diagnosis of doubtful lesions & for deciding adequate margin excision in case of malignancy. It is a rapid method for interpretation of surgical tissue for further operative management. Better communication of pathologist and surgeon will reduce interpretation error leading to high diagnostic accuracy & new improved standard of care.

Keywords: Frozen section, intra-operative diagnosis, histopathological correlation

Introduction
Intra-operative histology consultation was introduced for rapid examination of surgical specimens in 1905 by Dr. Louis B Wilson on request of Dr. William Mayo, now widely used for guidance of surgeons for surgical treatment (Wilson LB, 1905). The different modalities used for intra-operative consultation are squash smear cytology, frozen sections and fluid cytology. These investigations help to provide a preliminary diagnosis, enabling the surgeon to decide further management at the operating table. The ideal method for providing intra-operative diagnosis, apart from being rapid and accurate, should also allow tissue to be preserved for paraffin embedding and other ancillary studies if required. Intra-operative histology has long been applied as an effective diagnostic method for neoplastic as well as non-neoplastic lesions for multiple reasons namely organ identification, confirmation of clinical diagnosis of malignancy, determining per-operative extent of disease and margin status [1].
Frozen section plays an important role in surgical pathology and help the surgeon in intraoperative and preoperative patient management [2]. The technique was first used by William H Welch from John Hopkins Hospital in 1891 for intra operative consultation [3]. Later on 1905 this technique was further developed by Wilson and Mc carty for immediate

evaluation of frozen tissue [3]. Since then in 1959 after the development of cryostat frozen section become much easier and pathologist began to play a critical role in assisting and determination of the best approach during surgery [2, 4].
The main indication for frozen section is the immediate/ intra operative determination of nature of the lesion particularly differentiating between benign and malignant neoplasm to guide intra operative patient management [5, 6]. Apart from these, frozen section is also done to see the status of surgical margins, identification of lymph node metastasis in malignant lesions and confirmation of presence of representative samples for paraffin section diagnosis [2, 4]. Other indications are enzyme histochemistry, immuno-histochemistry and immuno-florescence [2].

Aim and Objectives
- Identification of tissue and unknown pathological processes.
- Evaluation of margins.
- Identification of lymph node metastasis
- Confirmation of presence of representative samples for paraffin section diagnosis
- Determine further surgical treatment protocols.
- Co-relation of frozen section diagnosis with routine histo-pathological diagnosis.

Type of study: Prospective study.
- **Materials and Methods**
- **Place of study:** The present study was carried at the department of pathology in collaboration with the departments of Gynaecology, Surgery and ENT in our hospital.
- **Design of study:** A diagnostic prospective study.
- **Duration of study:** 2 year.
- **Sample size:** Total 50 cases.

Inclusion criteria
- Any intra-operative fresh specimens sent in normal saline immediately on removal from body.
- Any surgical specimen including breast carcinoma tissue or margins, Oral cavity lesions such as tongue or buccal mucosa for margins, Thyroid specimens, Salivary gland tissue, renal tissues, pancreatic, liver tissues, Lymph nodes for metastasis, ovary, endometrium or cervical tissue, GIT tissue, Brain tissue, Retroperitoneal tissue, Testicular tissue.
- Minimum specimen size 2x1 cm.

Exclusion criteria
- Tissue with extensive necrosis or degeneration.
- Poor sampling of tissue.
- Confirm malignant lesion diagnosed by preoperative biopsy.
- Specimen size < 2x1 cm.

Sample type: Fresh specimen in normal saline.
Instrument used: SLEE semi-automated rotatory cryostat machine & MCM-ST medimeas semi-automated cryostat microtome.

Methods
The gross specimens was cut into thin slices and examined. Sections were taken from abnormal, suspected areas, or from firm lesions and placed in a mounting medium. The tissue was frozen immediately to - 20 degrees Celsius inside the Cryostat. After 10 minutes, when tissue was frozen, the chunk was inserted into clamping lever and was fixed. The frozen sections were cut on a Cryostat at the thickness of 5-7 micron and sections were stained by Hematoxylin and Eosin (H & E) stain. The sections were examined in correlation with appropriate clinical details. Report was immediately conveyed to the operating surgeon for further management. Subsequently, remaining tissue was fixed in 10% neutral buffered formalin, grossed and adequate representative sections were taken. The sections were then stained by H & E stain and examined. The frozen section diagnosis was correlated with the paraffin section diagnosis.

Result
In present study, 50 cases of frozen sections were included and correlated with histopathological diagnosis.

Table 1: Age-wise and Gender-wise Distribution

Age Group (Year)	Male (%)	Female (%)	Total (%)
≤ 30	00 (00%)	04 (08%)	04 (08%)
31-40	06 (12%)	12 (24%)	18 (36%)
41-50	01 (02%)	05 (10%)	06 (12%)
51-60	06 (12%)	09 (18%)	15 (30%)
>60	01 (02%)	06 (12%)	07 (14%)
Total (%)	14 (28%)	36 (72%)	50 (100%)

Above table shows that the majority of cases were in age group of 31-40 years (36%) with female predominance.

Table 2: Indication of Frozen section cases

Indication of Frozen section	No of cases
Primary Diagnosis / Typing of Neoplasm	33 (66%)
Evaluation of margins	17 (34%)
Total	50 (100%)

The primary indication of frozen section was primary diagnosis or typing of neoplasm (66% cases) and evaluation of margins for malignancy from resected specimen for malignant tumor (34%).

Table 3: Frozen section and histopathological Diagnosis of cases with Discordant and concordant cases

Organ/Site	Frozen section diagnosis	Histopathological Diagnosis	No of cases	Concordant cases	Discordant cases	Accuracy
Ovary (n=15)	Malignant Ovarian tumor? Mixed Germ cell Tumor	Mixed Germ cell - sex cord stromal tumor	01 (02%)	01	00	86.7%
	Benign Ovarian tumor	Sex cord Stromal Tumor - Theca Granulosa Cell Tumor	01 (02%)	00	01	
	Malignant Ovarian tumor? Granulosa cell tumor	Adult type Granulosa Cell Tumor of Right Ovary	01 (02%)	01	00	
	Malignant Ovarian Tumor	Sex cord stromal tumor	02 (04%)	02	00	
	No signs of malignancy	Normal ovarian tissue	01 (02%)	01	00	
	Benign ovarian cyst	Benign Dermoid cyst	02 (04%)	02	00	

	Haemorrhagic cyst	Haemorrhagic cyst	02 (04%)	02	00	
	Malignant Ovarian Tumor	Surface epithelial tumor - Serous cyst adenocarcinoma	02 (04%)	02	00	
	Benign mesenchymal ovarian tumor	Leiomyoma with myxoid degeneration	01 (02%)	01	00	
	Malignant ovarian tumor – Sex cord stromal tumor	Metastasis from epithelial malignancy	01 (02%)	00	01	
	Malignant secondary tumor in Bilateral Ovary	Metastasis in bilateral ovaries from epithelial malignancy - Krukenberg tumor	01 (02%)	01	00	
Breast (n=06)	Malignant Breast Lesion	Infiltrating Ductal Carcinoma	04 (08%)	04	00	83.3%
	Benign Spindle cell tumor of Breast	Benign Spindle cell Tumor of Breast	01 (02%)	01	00	
	Malignant Breast lesion	Benign Phyllodes Tumor of Breast	01 (02%)	00	01	
Testis (n=01)	Malignant tumor of Testis - Germ cell tumor of testis	Non Seminomatous Mixed Germ Cell Tumor - Predominantly Yolk Sac Tumor with Post Pubertal Teratoma of Left Testis	01 (02%)	01	00	100%
Endometrium (n=02)	Malignant lesion of Endometrium	Endometrial Intra epithelial Neoplasia (EIN)	02 (04%)	02	00	100%
GIT (n=03)	Malignant lesion	Moderately differentiated Adenocarcinoma of Colon	02 (04%)	02	00	100%
	Lipomatous tumor	Benign Lipomatous Tumor - Lipoma over epigastric Region	01 (02%)	01	00	
Retroperitoneum (n=2)	Benign soft tissue lesion	Benign Fibromatosis	01 (02%)	01	00	100%
	Angiomyolipoma	Angiomyolipoma	01 (02%)	01	00	
Thyroid (n=02)	Follicular variant of Papillary Thyroid Carcinoma	Follicular variant of Papillary Thyroid Carcinoma	01 (02%)	01	00	100%
	Benign Thyroid Lesion	Adenomatous Hyperplasia (Multinodular Goitre) with secondary changes	01 (02%)	01	00	
Salivary gland (n=01)	Benign Salivary Gland tumor	Benign Salivary Gland tumor - Pleomorphic Adenoma	01 (02%)	01	00	100%
Brain tissue (n=01)	Malignant Brain tumor? Glial tumor	Glial tumor - High Grade Astrocytoma	01 (02%)	01	00	100%
Margins (n=17)	All margins are negative for malignancy from resected specimen	All margins are negative for malignancy from resected specimen	17 (34%)	17	00	100%
Total (n=50)			50 (100%)	47	03	94%

Out of 50 cases, 15 cases were of Ovary, 06 cases of breast, 01 case from testis, 03 cases from GIT, 02 cases from endometrium, 02 cases from retro-peritoneal region, 02 cases from thyroid, 01 case from salivary gland, 01 case from brain tissue and 17 cases from margins for evaluation of malignancy. (Table 3).

Total discordant cases were 3, all were due to misinterpretation error. Overall diagnostic accuracy of frozen section was 94%. Out of which, 86.7% accuracy in ovary cases, 83.3% accuracy in breast cases and 100% accuracy in testis, GIT, thyroid, endometrium, salivary gland, retroperitoneal region, brain tissue and margins evaluation. (Table 3).

Most commonly received specimens were margins for evaluation of malignancy from resected specimen mainly breast, tongue and colon for malignant tumor (n=17) and all margins were negative for malignancy with 100% diagnostic accuracy. (Table 3).

Ovary was most commonly received specimen for frozen section for primary diagnosis of tumor. Out of 15 cases of ovary, all were correctly diagnosed except 2 cases (13.3% discordance) which were misinterpretation. One case was false negative for malignancy in frozen section and other was misdiagnosed. (Table 3).

Breast was 2nd commonest received specimen for primary diagnosis of tumor. Out of 06 cases, one case (16.7% discordance) was diagnosed false positive for malignancy in frozen section and other were correctly diagnosed.

Overall time interval between receptions of specimen to frozen section diagnosis was 30-35 minutes.

Few technical errors like difficulties in nuclear details due to freezing artefacts were observed.

Discussion

Frozen section is rapid method for guiding surgeons regarding further operative plans for better patient outcome and prognosis. It reduces chances of excessive removal of normal surrounding tissues by correct evaluating margins for malignant cells.

Frozen section requires cryostat machine, technical expertise for good sectioning and interpretation. Intra-operative frozen section is indicated in certain tumors to confirm malignancy; to evaluate surgical margins for malignancy in certain delicate tissues like tongue, oral cavity and neck region where excessive removal may affect the patient outcome and to evaluate lesion which was incidentally found during operative procedure.

In present study, overall diagnostic accuracy is 94% which is quite comparable with other studies. (Table 4).

Table 4: Comparative study of Diagnostic accuracy of frozen section by various authors

Study	Period of study (years)	No. of cases	Overall Diagnostic Accuracy (%)
Ahmad Z. *et al.* [7]	1	342	97.08
Patil P. *et al.* [1]	2	100	96.96
Misra S. *et al.* [3]	2	52	96.02
Shrestha S. *et al.* [8]	5	404	94.06
Agrawal P. *et al.* [9]	2	224	94.2
R.D.P. Silva *et al.* [4]	7	433	93.3
K. Chandramouleeswari *et al.* [10]	1	51	92
Present Study (2023)	2	50	94

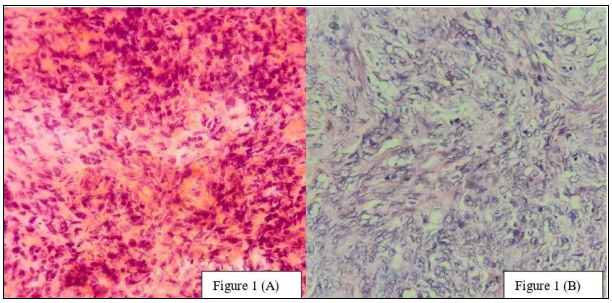

Fig 1: Krukenberg tumor of ovary (A) frozen section (B) histopathology

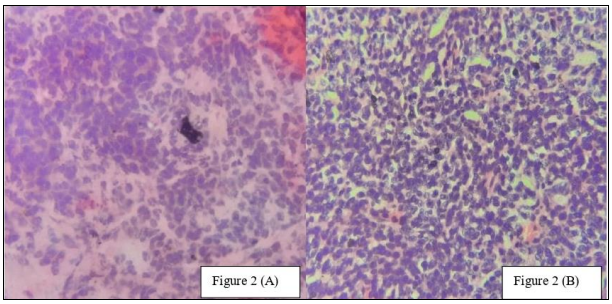

Fig 2: Granulosa cell tumor of ovary (A) frozen section (B) Histopathology

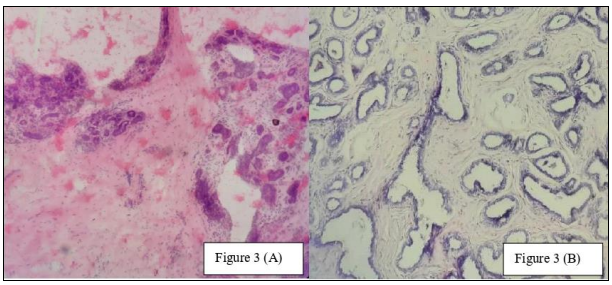

Fig 3: Benign breast lesion (A) frozen section (B) Histopathology

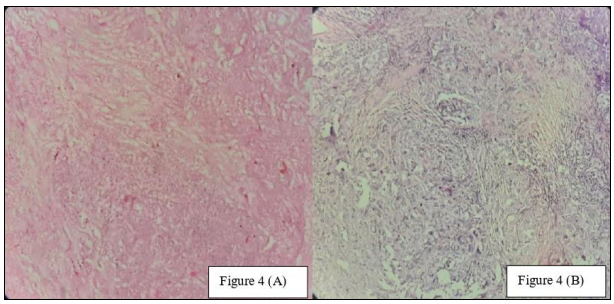

Fig 4: Carcinoma of breast (A) frozen section (B) Histopathology

Pinto *et al.* [11] studied 243 frozen section cases for ovarian tumors with accuracy rate of 98.55 for malignant tumors but only 78.6% for borderline tumors. Use of frozen section for tumor grade is less sensitive with accuracy of only 88.6% in 260 endometrial carcinomas studied by Quinlivan JA *et al.* [12] The accuracy rate is generally very high in determination of margin clearance may be quite costly cannot reliably eradicate positive final margin [13]. The accuracy rate in present study for ovary tumor is 86.7, for breast tumor is 83.3; for endometrial tissue, brain tissue, thyroid, testis, salivary gland, GIT, retroperitoneal tissue and margin evaluation are 100%.

Conclusion

The frozen section diagnosis is very useful for intra-operative diagnosis of benign and malignant lesions & for deciding adequate margin excision in case of malignancy. It is a rapid method for interpretation of surgical tissue intra-operatively for further operative management. Better communication of pathologist and surgeon will reduce interpretation error and lead to high diagnostic accuracy.

This project has successfully achieved its multifaceted objectives, shedding light on the invaluable contributions of frozen section techniques in intraoperative histology consultation. The findings not only enhance our understanding of these techniques but also offer practical guidance for surgeons in making informed decisions during surgical interventions.

Challenges and Recommendations

While the study encountered challenges such as freezing artifacts impacting nuclear details, these were recognized as areas for improvement. Proper technical training of staff is required to reduce technical errors like freezing artifacts, chattering and folding of section.

Recommendations include continuous refinement of techniques and technological advancements to further enhance accuracy.

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Conflict of Interest

Not available.

Financial Support

Not available.

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