



Intra Operative Cytological Examination as a Diagnostic Tool for Carcinoma Breast Comparing With Frozen Section

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ABSTRACT

Introduction: *Intra operative cytology and frozen section in carcinoma breast*

Materials and Method: *FNAC proven surgical specimens of breast malignancies form the Department of surgery during the period 3years. Total of 200 cases with relevant clinical details, gross morphological features of the lesions were observed and recorded. Then specimens for cytological examination and frozen section study were collected, from the suspected focus of malignancy such as infiltrative and irregular gray white areas. Nuclear grading was done on cytological smears. Cytology smears compared with frozen sections. Study was approved by the institutional ethical committee.*

Result: *Gross examination and intra operative cytology together gave an accuracy rate of 100% in malignant breast lesions. With frozen section alone, the accuracy rate was 96%.*

Conclusion: *Intra operative cytology adds confidence to frozen section study. Intra Operative consultation by cytological examination was an easy procedure to perform and requires less time compared to frozen section and is of great value in areas where frozen section facility not available.*

Keywords: *Intra operative cytology, frozen section, breast malignancy.*

Introduction

Intra operative consultation by frozen section is a very important aspect of practice in surgical pathology that is often guiding the surgeon's hand. Supplementing or replacing frozen section by cytological Touch, Scrape, or Crush preparations has been in use in neuropathology for many years and is recently receiving attention in other areas of pathology as well. Breast malignancy is one such area where intra operative diagnostic technique act as valuable tool for management.

Objectives

- 1) To evaluate the role of intra operative cytology in diagnosis of malignant breast lesions.
- 2) To compare accuracy of intra operative cytology preparations and frozen sections in malignant breast lesions.
- 3) Study frozen sections and analyze its role in intra operative diagnosis of malignant breast lesions.

Materials and Methods

Case Selection

Study material were collected from FNAC proven surgical specimens of breast malignancies from the Department of surgery during the period January 2005 to December 2006.

Sample size

A total of 200 specimens were collected and examined.

Inclusion Criteria

All patients undergoing elective surgery with FNAC proven breast malignancy.

Exclusion Criteria

Patients who underwent lumpectomy for benign/suspicious for malignancy were excluded.

Study Design

After collecting relevant clinical details, gross morphological features of the lesions were observed and recorded. Then specimens for cytological examination and frozen section study were collected, from the suspected focus of malignancy such as infiltrative and irregular gray white areas.

The methods of collecting cytological specimen are

- 1) Imprint method
- 2) Scrape method
- 3) Scrimp method

Touch imprint method was done by gently holding the tissue against a glass slide.

In scrape method cut surface of the lesion is blotted with wet cotton to remove excess blood and exudates. It was then scraped with one end of glass slide forming an acute angle with the surface to be sampled. The pressure applied for scrapping and number of scraping movements needed to collect the material on the under surface of one end of slide varied with the consistency of the specimen. The scraped material accumulated at one end of the glass slide was pressed against another slide and moved in opposite direction. This leads to loosening of the architectural pattern of the cells and they are distributed as a single layer on the slide³. The scrimp method is performed by scratching the tissue with blade and then smearing the material obtained on to the slide. Scrape and scrimp methods are mainly used for firm or sclerotic lesions.

The materials obtained by these methods were fixed in 80% isopropyl alcohol for 2 minutes and then stained with Hematoxylin and Eosin or Papanicolaou methods. In thirty cases the smears were air dried and rapid Giemsa was done (Fig: 4).

The entire procedure of preparation, staining and reporting was completed within 15 minutes. The criteria used for reporting were similar to those for aspiration cytology.

Selection of tissue for frozen section

Small thin portions of tissue, generally not more than 0.5 x 0.5 x 0.3cm freeze best. Fat should not be included in the specimen for obtaining better section. When partially frozen, the block can be inverted on the shelf to create a flat surface. For breast and fatty tissue temperature is kept very low as -200C.

Section cutting

The sections are cut with 5 micrometer thickness in a cryostat. The slide is then immediately placed in 80% isopropyl alcohol. The entire process of preparation staining and reporting takes about 20-30 minutes.

Staining

The slides are stained with hematoxylin and Eosin. Cytology slides can also be stained by rapid Giemsa method, in these situations the slide is air dried and then stained.

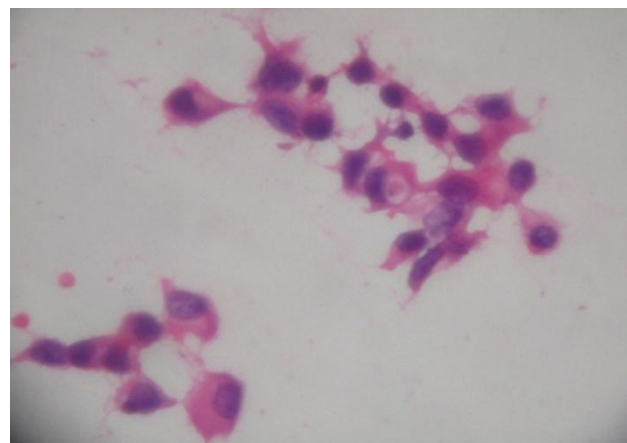
Observations

In the present study, 200 surgical specimen of carcinoma breast were studied. Cytological specimen and frozen section prepared intra operatively were analysed along with gross features of the lesion and relevant clinical details. Age group with carcinoma breast in our study was between 45-49 yrs (20%). Out of 200 cases 195 cases were females and 5 cases males. Both right and left breasts were involved in an almost equal proportion. Gross features of the tumor were noted and recorded. The features which suggested malignancy included hardness of lump with fixity to adjacent tissue. In advance cases skin fixity was noticed with puckering or ulceration of the skin. Maximum tumor size was recorded as it is a prognostic

indicator. Tumor size along its greatest dimension was noted. In 140 cases the size was in between 2 to 5cm. In 34 cases size was more than 5cm and in 26 cases size was less than 2cm in greatest dimension. Nuclear grading was done for all cases.

Cytological Nuclear Grading Table No.1

Nuclear grade I	- Nuclei similar to those of normal duct epithelium - Minimal nuclear enlargement - Round smooth nuclear contours - Fine granular chromatin - No obvious nucleoli
Nuclear grade II	- Nuclei approximately twice that of Grade I - Smooth nuclear border - Uniform chromatin - Small nucleolus
Nuclear grade III	- Marked anisonucleosis - Three fold variation in nuclear diameter - Irregular nuclear contours - Increased hyperchromasia - Coarse chromatin with chromatin clearing - Macro nucleoli 9.

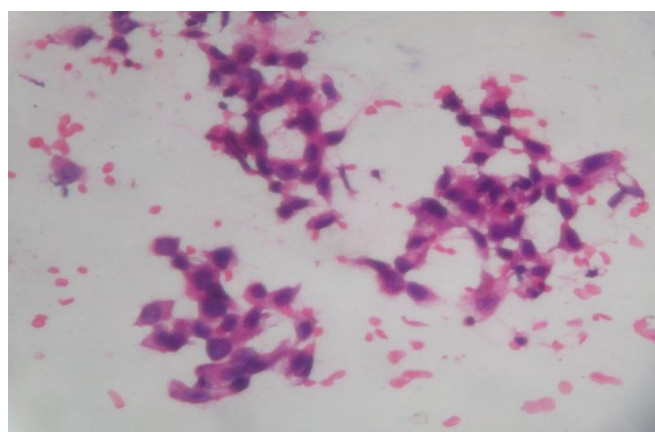
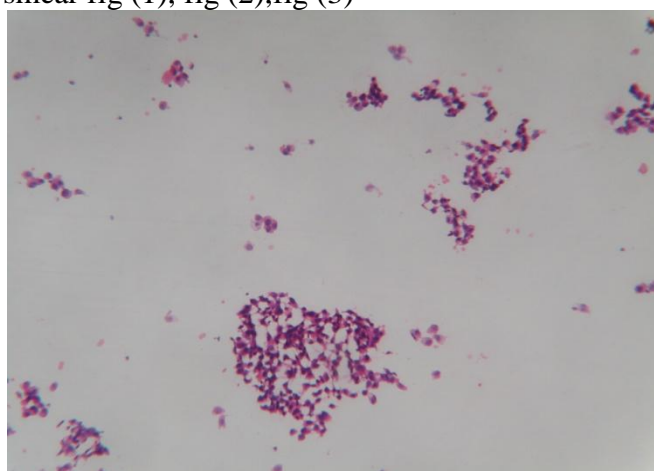


Nuclear Grade in Present study Table No.2

Grade	Number of Cases
Grade I	17 Cases (8.5%)
Grade II	162 Cases (81%)
Grade III	21 Cases (10.5%)

In both cytological and histopathological examination maximum number of cases showed nuclear grade II. Cases showing nuclear grade III were more in cytological smears than in histopathology sections.

Carcinoma breast with nuclear grade II scrape smear fig (1), fig (2),fig (3)



Microscopy

In the case of cytology smears, the features studied are –

- Arrangement of cells
- Staining of cytoplasm
- Amount of cytoplasm
- Nuclear cytoplasmic ratio
- Nuclear membrane
- Characteristics of chromatin
- Presence of nucleoli
- Background

In the case of frozen section the features studied were

- Architectural pattern
- Infiltration to adjacent tissue
- Cell morphology
- Background

Imprint smears

They showed less cellularity especially in small lesions and also in malignancies with sclerotic stroma. Malignant cells were seen scattered singly or in small clusters with moderate to abundant intact

cytoplasm. Nuclei were eccentrically or centrally placed which were pleomorphic with irregular nuclear membrane, clumped chromatin, and prominent nucleoli. Mitotic figures were seen. Bizarre cells and multinucleated cells were seen in high grade lesions. 40 (20%) of cases showed intra cytoplasmic lumina formation)

Scrape smears

There were good cellular yield and architectural pattern of the tumor could be appreciated in areas along with cell morphology^{13,36}

Scrimp method

This method was used in sclerotic and small lesions. There were good cellular yields with cells in sheets and scattered singly

Frozen section

In frozen sections the infiltrative pattern could be well appreciated. In 76 cases the sections showed freeze artifact but diagnosis was possible. Eight cases showed complete necrosis tissue which was difficult for sectioning. In this situation it was difficult to report on frozen section alone.

Discussion

Histological intra operative examination dates back to 1891 and from 1920 onwards intra operative imprint smear cytology was also in use^{1,2}. Intra Operative morphological diagnosis is reliable and quick. Present study is an attempt to understand the extent to which frozen sections and intra operative cytological examinations are useful in this aspect.

In present study, the most frequent age group with carcinoma was between 45 and 49yrs. Majority of the patients presented with a tumor size more than 2cm in size or in advanced stage with skin, nipple and areola involvement at the time of surgery.

The low cost benefit ratio in per operative diagnosis is the most widely claimed advantage of intra operative cytology over frozen section study. Gross inspection of the growth was done prior to collection of the specimens. With a combination of naked eye examination and intra operative cytology, a high degree of diagnostic accuracy could be obtained¹².

Mode of obtaining cytological material depends on

the consistency of lesion. Scrape method was preferred over imprint method in most of the cases because it yields more cells and was giving few tissue fragments from which architectural pattern could be made out. The number of scrapings and the amount of pressure to tumor^{3,6,10}. be applied depend on the consistency of Imprint method was yielding very few cells in case of small lesions and malignancies with sclerotic stroma. It is very difficult to report on such sliders. But in necrotic soft lesions imprint method was preferred over scrape cytology because with scrape method there is too much of background debris, and thickness of preparation is also more. Then it is very difficult to interpret the cell morphology. But in imprint smears, with fewer materials, cell morphology is very clearly detected. In case of sclerotic and small lesions scrimp method was preferred because it yields enough cellularity^{3,12}.

The method of staining used was Hematoxylin and Eosin in most cases as it was easy to compare with the frozen section specimens. Even though the cell morphology was well appreciated in cytology smears, infiltrative and growth patterns of the tumor were well observed in frozen sections. The cytology smears can easily be prepared and stained. There is no need for an experienced technician.

In the present study, the diagnosis accuracy rate for per operative gross and cytological examination together was 100%. For frozen section alone, the accuracy rate was 90%. The diagnostic accuracy of the frozen section technique varies from 93.13 to 100% in literature, while for cytology it is 95.33 to 100%, in malignant breast lesions. 100% accuracy rate could be achieved by combining both frozen section and cytological examination.

In frozen section studies 8 cases could not be diagnosed. This was due to improper sampling that yielded completely necrotic tissue.

The accuracy of the diagnosis in intra operative cytology smears depend on the combination of factors like gross inspection of the tumor and assistance of a competent Cytopathologist. The time interval taken for the final diagnosis was more in frozen section studies when compared to that of

intra operative cytology smears.

Frozen section preparation can be avoided at any time when definite features of malignancy can be offered by macroscopic and cytological examination.

It is a simple, inexpensive method not requiring any special instrument or a technician. Immunohistochemistry for estrogen and progesterone receptors can also be performed on the cytological smears^{4,5}

Prognostic assessments with nuclear grade and tumor size can be done per- operatively. Nuclear grading of cytology smears is comparable with histopathology nuclear grade and is an important prognostic indicator¹¹.

Conclusion

- In the present study incidence of carcinoma breast was highest among the age group 45-49yrs.
- 140 (70%) cases had a tumor size of 2-5cm at the time of surgery.
- Intra operative cytology and frozen section diagnosis were compared with that of permanent paraffin sections.
- Results obtained by Intra Operative Cytology showed good agreement with that of histological diagnosis and nuclear grading in malignant breast lesions.
- Gross examination and intra operative cytology together gave an accuracy rate of 100% in malignant breast lesions. With frozen section alone, the accuracy rate was 96%.
- Intra operative cytology adds confidence to frozen section study especially in lesions with large necrotic area and freeze artifact.
- Intra Operative consultation by cytological examination was an easy procedure to perform and requires less time compared to frozen section and is of great value in areas where frozen section facility not available.

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