

Diagnostic efficacy of imprint cytology and frozen section of breast lesions

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Abstract

Objective: To perform imprint cytology of operable breast masses and evaluate its accuracy in relation to histopathological diagnosis after H & E staining and to perform frozen section on operable breast masses and evaluate its accuracy after histopathological diagnosis with H & E staining.

Materials and Methods: A total of 82 surgically resected specimens were examined by both imprint cytology and frozen section and compared with histopathological sections which were considered as gold standard.

Results: Out of 82 cases 4 taken were inadequate. Total 78 cases subjected to imprint cytology which diagnosed all 43 benign lesions correctly and out of 35 malignant lesions 29 were diagnosed correctly with 6 FN results and no FP results were seen. Imprint cytology showed sensitivity, specificity and accuracy of 100%, 82.86% and 92.31% frozen section diagnosed all 43 benign lesions correctly and out of 35 malignant lesions 33 were correctly diagnosed with 2 FN results and no FP results were seen.

Frozen section showed sensitivity, specificity and accuracy of 100%, 94.29% and 97%.

Correlation of both imprint and frozen section alone showed 45 benign lesions and out of 33 malignant lesions 29 were correctly diagnosed with 4 FN results and no FP results were seen. Imprint cytology and frozen section combined efficacy showed sensitivity, specificity and accuracy of 91.84%, 100% and 94.87%.

Final correlation of both imprint cytology and frozen section with HPE showed significant association.

Conclusion: FS is superior to imprint cytology when compared with gold standard HPE in intraoperative diagnosis of breast mass lesions.

Keywords: Frozen section, Imprint cytology, Breast.

Introduction

Breast is one of the important organ in human being and in female, they represent motherhood, infant nutrition and sex. Breast lesions commonly affect female and form a spectrum. This spectrum consists of both benign and malignant tumors.

Breast carcinoma is the second most common malignant tumor and one of the leading causes of death in women.¹ The incidence of breast cancer is rising in the world especially in developing countries such as India. It accounts more than 1,000,000 cases occurring worldwide annually.¹

According to the National Cancer Registry Programme report on time trends in cancer incidences rates (1982-2005) of Indian Council of Medical Research (ICMR), the estimated breast cancer cases in India in 2010 is 90,659. Indian's National Health Profile 2010 predicted that by 2020, breast cancer will overtake cancer.² The incidence of breast in Kolar district is around 6.41%.³

Breast cancer increases the anxiety, cosmetic concern, loss of symbol of femininity, fear of death due to cancer and decision of therapy become a challenge.⁴ This has led to evolution of many diagnostic procedures for breast lesions apart from clinical examination they are.⁴

1. Mammography
2. FNAC
3. Core needle biopsy
4. Incisional biopsy-for large tumors
5. Excisional biopsy for tumors <2cms
6. Imprint cytology

7. Frozen section.

A palpable breast lump is a common diagnostic problem to both attending clinician and pathologist. Aim of diagnostic procedure is should be simple, reliable, reproductive, less traumatic, cost effective, less time consuming and its diagnostic accuracy. Mammography helps in detection of malignant neoplasms non-invasively.⁴ Approximately 20% of palpable tumor remains undetected.⁴ Excisional or incisional biopsy of breast lumps also a diagnostic procedure which is time consuming.

The triple assessment consisting of clinical evaluation, mammography and fine needle aspiration cytology has been routinely practiced and it is an alternative to conventional open biopsy in the pre-operative diagnosis of breast lumps.⁵ It is simple, reliable, reproducible, less traumatic, cost effective, less time consuming and its diagnostic accuracy has been reported to reach 100%.⁵

FNAC is challenging diagnostic tool which is performed preoperatively with studies mentioning its efficacy. FS is another diagnostic procedure which is usually done intra-operatively and also considered as therapeutic decision making procedure.⁶ IC is another diagnostic tool which is used intra-operatively and is considered better than FNAC.⁶ To increase diagnostic accuracy the combined use of IC and FS are recommended.⁶ The choice of diagnostic procedure varies according to the person evaluating the case. The present study is undertaken to find out the diagnostic accuracy of imprint cytology (IC) and frozen sections (FS) which varies from center to center.

Objectives of the Study

1. To perform imprint cytology of operable breast masses and evaluate its accuracy in relation to histo-pathological diagnosis after H & E staining.
2. To perform frozen section on operable breast masses and evaluates its accuracy after histo-pathological diagnosis after H&E staining.
3. Final correlation between frozen section and imprint cytology together with gold standard histopathology diagnosis.

Material and Methods

This study was done in the department of pathology, SDUMC, Kolar in coordination with department of surgery. Over a period of 2 years a total of 82 patients having a palpable breast lump were considered for the study and were examined by both IC and FS and were co-related with histo-pathological sections which were considered as gold standard.

Imprint Slide Preparation

Clear glass slide was touched gently on the cut surface of the specimen at several places. Three slides were fixed immediately in ethanol for methylene blue, pap and H&E stain. Another slide was air dried for giemsa stain. Methylene blue stained slide examined immediately to assess cellularity and for the possible diagnosis.

Frozen section was done using Leica CM 1100 cryostat.

Procedure: Specimen was sent for frozen section in normal saline. Tissue was measured then it was sectioned at an interval of 1cms, tissue was examined for obvious features.

The tissue was frozen as quick as possible in order to avoid ice crystal formation resulting in artifact and poor morphological preservation.

Cryostat was kept ready at -20 °C to -30 ° C beforehand. Tissue from representative area was taken and full from kept on the slide with proper orientation. OCT was added till it covers the tissue and frozen.

OCT frozen section embedding medium was placed on a cryostat object disk.

The frozen specimen was positioned in the center of the object disk and the disk was placed on the cryobar in the cryostat to begin the quick freeze process.

As the OCT freezes it turns from a clear gel to white solid substance.

Before the disk is frozen solid OCT was added to cover the top of the specimen and quickly a heat extractor was placed on top of the specimen.

Object will be placed on disk in the microtome by object disk holder and the screw will be tightened or clamped.

The ratchet was engaged on the micrometer gear, trimmed until tissue appears and the first two or three sections were discarded.

Cutting was done slowly and continued until a full tissue section were obtained.

The slides were lowered onto the blade, keeping the slide parallel to the section. As the tissue comes into contact

with the slide the OCT and tissue will melt causing the tissue to adhere to the slide.

The slides were placed in fixative of 95% ETOH for 30 sec and sections were stained with rapid haematoxylin and eosin stain (H&E), Papanicolaou method (PAP) stain.

On IC cellular details, pattern of arrangement of cells, nuclear morphology and other specific nuclear details with respect to particular tumor was analyzed.

FS was analyzed based on tissue architecture, cellular details, mitotic activity, necrosis and lympho vascular invasion.

Both morphological features of IC and FS were compared with histo-pathological features.

Rest of the tissue was fixed in 10% formalin and processed in Leica Histokinete. Processed tissue was embedded in paraffin wax and blocks were made.

The stains used in the study include:

Papanicolaou method (PAP)⁷

Procedure: fix the smear in 95% alcohol for 15-30 minutes

Hydrate in 70% alcohol and 50% alcohol for 2 minutes

Rinse in water, 1 minute

Stain in Harri's haematoxylin, 5 min

Rinse in water 2 min

Differentiate in 0.5% aqueous hydrochloric acid 10 seconds

Rinse, bluing done in water 2 min

Dehydrate in 50% 70% 90% alcohol for 2 min each

Stain in OG 6, 2 min

Rinse inn 95% alcohol for 2 min each

Stain in EA50, 3 mins

Rinse in 95% alcohol 1min

Rapid haematoxylin and eosin stain (H & E)⁷

Fixation in alcohol for 20 secs

Rinse in water

Stain in Harri's haematoxylin for 1.5 min to 2 min

Rinse in water and drip in 1% acid alcohol and again rinse in water

Stain in eosin for 10 sec to 15 sec

Rinse in tap water

Dehydrate clear and mount in DPX

Standard haematoxlin and eosin (H & E) for paraffin section⁷

Dewax sections, hydrate through graded alcohol to water

Remove fixation pigments if necessary

Stain in Harris haematoxlin for 10-20 min

Wash in running tap water until sections blue (for 5 min or less)

Differentiate in 1% acid alcohol for 5-10 seconds

Wash well in tap water until sections for again blue (for min or less)

Stain with 1% eosin for 1 min

Wash in running tap water for 1-5 min

Dehydrate through alcohol clear and mount

Methylene blue stain (3-4 mins of timing)⁷

Slides placed in 90% alcohol for fixations

Keep in a slide rack flood with 1% methylene blue and leave for ½-1min

Rinse rapidly in water transfer to pad of filter paper and blot firmly

Flood with xylene and mount in DPX.

Total 82 cases comprising 47 benign lesions and 35 malignant lesions on HPE. Out of 82 cases 4 cases were inadequate in both (IC and FS) smears and were excluded for statistical analysis. The distribution of subjects according to clinical diagnosis is mentioned in Table 4. Comparison of IC with gold standard HPE showed that out of 78 cases 43 were benign lesions which were correctly diagnosed by IC and out of 35 malignant lesions 29 were correctly diagnosed and 6 false negative were seen. There was no false positive result in our study which was statistically significant.

In our study IC compared with gold standard HPE showed the following sensitivity, specificity, PPV, PV and accuracy respectively 100%, 82.86%, 87.76%, 100%, 92.31% and are represented in Table 6.

Association of both IC and FS with HPE showed 43 benign lesions which were correctly diagnosed but out of 35 malignant lesions 2 were false negative and 4 cases showed both benign and malignant component. There was significant association between HPE and IC and FS.

Discussion

Breast carcinoma is the second most common malignant tumor among Indian women.¹ Intraoperative procedure like IC and FS have an important role despite the widespread popularity of aspiration cytology in cases of difficult cytology, evaluation of lumpectomy margins, intraoperative nodal status.

In our study benign tumors were commonly seen between less than 30 years of age group (18-30 years) and patients with more than 40 years frequently presented with malignant tumors.

Our study observations are similar to the study of Khudier et al⁸ which showed most of the benign tumors were seen in second decade and most of the malignant tumors were seen in fourth decade of life.

IC is technique which is accurate, simple, rapid, and cost-effective, does not require any special instrument and less time consuming and gives rapid tissue diagnosis. It is a touch preparation in which tissue is touched on the slide and it leaves behind its imprint in the form of cells on glass slide. Imprint cytology was first introduced by Dudgeon and Patrick in 1927; they examined fresh tissue by the wet film method on breast.¹

This is used for examination of individual cells and preserves the histological pattern. The accuracy of IC has been increased over the years both in breast pathology and in other body sites, the average accuracy of (90-94%) in the past has reached (97-98%) recent years.⁹

The sensitivity, specificity, and accuracy of various studies as shown in Table 1.

Literature shows following points to improve the accuracy of IS²⁰⁻²² a) The tissue surface should be flat for taking smears of imprint. b) No portion of fat should protrude from edges as this may lead to smudge the imprints. c) If first imprint smear contains excess of tissue

fluid and blood and the subsequent imprints gives better cytological results.

To obtain imprint nearest to one cell thickness, the amount of pressure applied at the time of imprinting should be varied.^{21,22} Benign lesions usually requires more pressure in order to obtain sufficient cells for diagnosis while malignant tumors get imprinted more easily.² Imprinted piece or tissue should be flat and no fat should be left extruding from the surface.²³

The study done by tribe (1965) showed that gross examination of breast lump helps to distinguish between benign and malignant tumors in 95.1% cases.²⁰ Suen et al, (1978) examined 473 breast lesions and report that grossly malignant lesions requires imprint cytology which provides rapid intraoperative diagnosis.²¹ Singh et al., 1982 showed that imprint diagnosis gives 100% results when combined with clinical examination and gross appearance.²² Scucchi 1997 described the advantages of IC with 2250 cases comparing with FS and they are as follows²⁴. a) It is rapid with same accuracy rate with frozen section. b) It has excellent preservation of cellular details devoid of freezing artifact. c) It helps in identifying focal macroscopically undetectable neoplastic lesions in larger tissue fragments. d) Determine adequacy of small surgical samples for definitive histological examination. e) Ability of wider sampling. f) It helps in sparing tissue for special investigation such as receptor studies, electron microscopy, immunocytochemistry and other biological studies.

The FS technique is one of reliable and accepted method in intraoperative consultation for more than 100 years.²⁶ It is used to identify the nature of the lesion, to evaluate the involvement of surgical margins in malignant tumors and to determine the adequacy of diagnosable material. The main indication of FS is to determine the tissue sampled is malignant or benign.¹⁵ The overall accuracy of FS reported in different studies vary 91.5 to 97.4%²⁵ as shown in Table 2.

The earliest use of FS technique is attributed to Dr. Welch of Johns Hopkins Hospital in 1891 on benign breast tumor removed by Dr. Halstead.²⁶ Cullen described a rapid method of making permanent specimens from FS in 1895.²⁶ Wilson of Mayo Clinic, developed FS staining method (methylene blue) in 1905.

Hazard and Stevenson in 1948 introduced a technique which was compared to modern procedures using the cryostat. In this technique the fresh specimen was fixed with alcohol. The fixed block is then frozen between pieces of dry ice and cut at 10-15µm with a microtome knife.^{26,27}

Breast cancer is observed rarely below the age of 40 but the proportion of tumors classified as such in young breast cancer cases are also similar.¹ It may depend on these risk factors including geographical, culture, lifestyle, reproductive variables.¹

In our study 97.6% female presented with breast lesions compared to male 2.4% which is similar to the study done by Ramraje et al² 2012. In their study 96.6% of females presented with breast lesions compared to males 2.4%.² Study done by Harnish et al also represents same showing

(60%) females are more commonly affected compared with (40%) males.¹⁹ In our study clinically 45 patients presented with benign lesions and 37 patients presented with malignant lesion. In a study by Ramraje et al² showed out of 90 cases 45 cases clinically presented with benign lesion and rest with malignant lesions.²

In the present study a total of 82 cases showed 47 benign lesions and 35 malignant lesions which is similar to Khudier et al were a total of 110 cases consisting of 81 benign lesions compared and 29 malignant cases were studied.⁸ Similar to Tribe et al our study included fibroadenoma and IDC.²⁰

Gross features of our study showed benign lesions small, firm, well circumscribed with cut section showing grey white with whorled appearance with cystic change and malignant lesion were large, firm to hard, irregular shape, gritty to cut. Similar to the study of Ramraje cut section showed hemorrhagic, cystic, necrosis.²

In the present study IS were categorized into two i.e. benign and malignant lesions which showed following features: Benign lesion-thin, uniform, hypocellular, found in clusters whereas, malignant lesions were showing thick, hypercellular arranged in sheets, clusters. These observations were similar to the study of Ramraje 2012.²

In our study a total of 82 cases were taken for both touch IS and FS. Finally these were compared with gold standard histo-pathological examination (HPE). Out of 82 cases 4 cases were inadequate in both IC and FS due to sampling error, so these cases were excluded for statistical analysis. The literature shows limitations of both imprint cytology and frozen section. Imprint cytology shows inadequacy smear rate ranging from 2.95 to 10%.¹² Limitations of FS slides were observed suboptimal or inadequate because of necrosis, hemorrhage, calcification, non-representative sampling or other technical factors.¹⁴

Present study is similar to the study done by Khudier et al⁸ showed unsatisfactory imprint smears were seen in 8.2% (9 cases) comprised of fibroadenoma, fibrocystic disease, fat necrosis, gynecomastia. Inadequacy in case of fibroadenoma could be attributed to the excess of fibroadipose tissue, fibrocystic disease showed only inflammatory cells, fat necrosis showed only fat cells, in infiltrating ductal carcinoma (NOS) and phyllodes tumor because of excessive fibrosis obscuring the cytological details. It could also be due to technical or procedural errors which are commonly documented limitations of both the procedures.²

The high inadequacy rate described by various authors is attributed to desmoplasia, technical error and inexperience.¹² Ceserni et al²⁸ explained the errors in frozen section were due to a

a) Misinterpretation b) Poor quality of frozen section c) Sampling error during sectioning d) Ignorance of macroscopic findings e) Lesions difficult to interpret (DCIS)

Comparison of imprint cytology with HPE which showed out of 78 cases 43 were benign lesions which were correctly diagnosed by IC and out of 35 malignant lesions 29 were correctly diagnosed and 6 false negative were seen.

There was no false positive in our study which was statistically significant.

In our study imprint cytology showed sensitivity, specificity, PPV, NPV and accuracy were 100%, 82.86%, 87.76%, 100%, 92.31%.

Total 6 cases of imprint cytology showed false negative (Paraffin sections of these cases showed IDC, Medullary carcinoma with DCIS, phyllodes, DCIS, Microinvasive carcinoma and ILC) these tumors were characterized by low cellularity.

Ramraje et al² 2012 described that carcinoma with more fibrous stroma may yield less cells which can be mistaken for benign lesions in imprint cytology.²

Comparison of FS with HPE: Out of 78 FS diagnosed correctly 43 benign and 35 malignant lesions were diagnosed. Of the 35 malignant cases 33 were correctly diagnosed with 2 false negative (FN) results and no false positive results were seen. FS showed sensitivity, specificity, PPV, NPV shows 100%, 94.29%, 95.56%, 100% and 97.44% respectively. The 2 false negative results HPE showed DCIS with desmoplastic stroma and malignant phyllodes which was due to sampling error.

A study of large retrospective analysis of frozen section diagnoses documented that diagnostic errors related to intraoperative consultations can be divided into the following 4 groups; those resulting from interpretation (57%), microscopic sampling (24%), gross sampling (9.5%), and lack of communication between the pathologist and surgeon (9.5%).²⁹

Literature show false negative are often associated with diagnostic discrepancies ranging from 0.4-2.56%.

The limitation of FS diagnosis includes selection of small pieces of tissue for intraoperative diagnosis especially for tumor like phyllodes and adenofibroma, atypical hyperplasia and DCIS and microinvasive carcinoma.³³

Comparison of both imprint cytology and frozen section alone showed out of 78 cases 45 benign lesions were correctly diagnosed. Out of 33 malignant lesions 29 were correctly diagnosed. 4 false negative were seen with no false positive results. Sensitivity, specificity, PPV, NPV and diagnostic accuracy were 91.84%, 100%, 100% 87.88% and 94.8%. In those cases (HPE diagnosis showed DCIS with medullary carcinoma, microinvasive carcinoma, ILC and IDC). As earlier quoted limitations of IC and FS.³⁰⁻³²

4 cases of IC which were false negative, on doing FS 3 tumors turned out to be malignant; by this we can tell that frozen section is preferable over imprint for intraoperative consultation however cytology is better than FS.³³

Another case which was false negative by FS may be due to sampling error as quoted in literature.²⁸ This highlights the importance of FS over IC. Only 4 cases showed both benign and malignant component in imprint cytology and frozen section when compared with gold standard HPE, so a reasonable statistical analysis could not be done.³⁴

Table 1: Showing sensitivity, specificity and accuracy of other studies

Authors	No. of cases	Sensitivity	Specificity	Accuracy	Deferrals
Scopa ¹⁰ (1990)	82	100%	100%	100%	4 (4.9%)
Khanna ¹¹ (1991)	86	98.4%	100%	98.8%	6(6.9%)
Veneti ¹² (1996)	351	97.1%	99.4%	98.3%	7(1.9%)
Albert ¹³ (2000)	173	96.5%	90%	95.4%	12(6.9%)
Bolkainy ¹⁴ (2008)	122	92.2%	93.3%	92.5%	8(6.5%)
Khudier ⁸ (2009)	107	96.3%	100%	98.9%	4(3.9%)

Table 2: Shows sensitivity, Specificity and accuracy of other studies

Authors	No of cases	Sensitivity	Specificity	Accuracy
Abbasi ³¹ (2012)	200	92.3%	96%	90.3%
Bolkainy ¹⁴ (2008)	128	100%	100%	100%
Haeri ²⁹ (2002)	125	92.4%	100%	95.4%

Table 3: Site of breast lesion

Site	Frequency		Percent
	Left	Right	
	45	37	54.9
		82	45.1
			100.0

Table 4: Distribution of subjects according to clinical diagnosis

Clinical diagnosis	Frequency	Percentage
Carcinoma breast	37	45.12%
Fibroadenoma	37	45.12%
Fibrocystic disease	4	4.90%
Breast abscess	2	2.43%
Mastitis	2	2.43%
Total	82	100.00

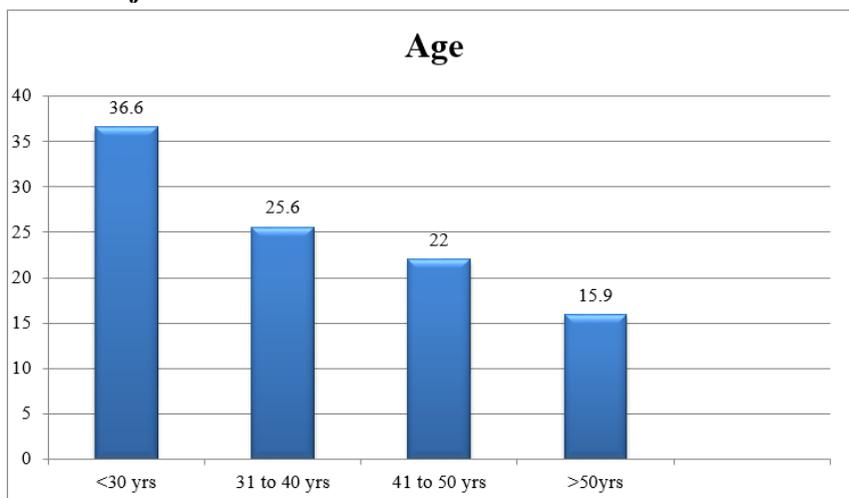
Table 5: Gross findings: Tumors were divided in two groups based on following findings

	Benign lesions	Malignant lesions
Size	Small	Large
Consistency	Firm	Firm to hard
Appearance	Well circumscribed	Poorly circumscribed
Cut section	Grey white appearance	Gritty to cut with grey white appearance
Surrounding areas	May be cystic change	Hemorrhagic, necrosis, cystic

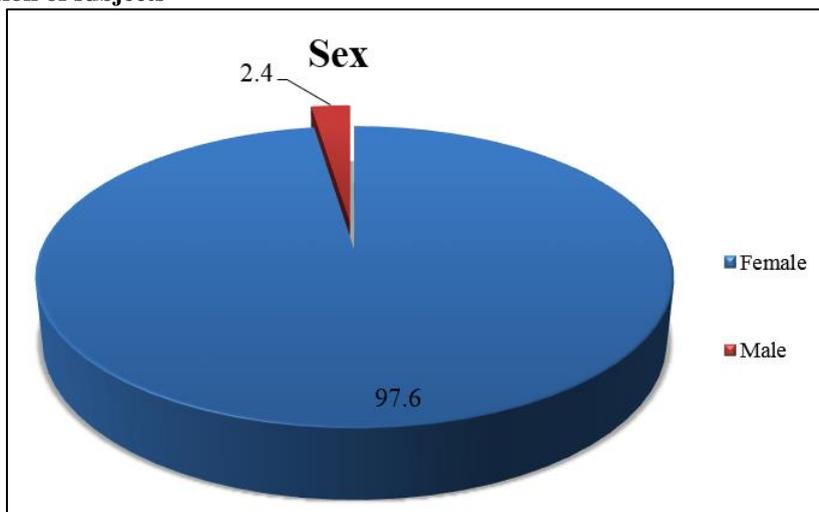
Table 6: Diagnostic ability of Imprint cytology in breast lesions with respect to gold standard HPE

		HPE		Total	X ² , df, P value
		Benign	Malignant		
Imprint cytology	Benign	43	6	49	56.715,1, 0.0001**
	Malignant	0	29	29	
Total		43	35	78	

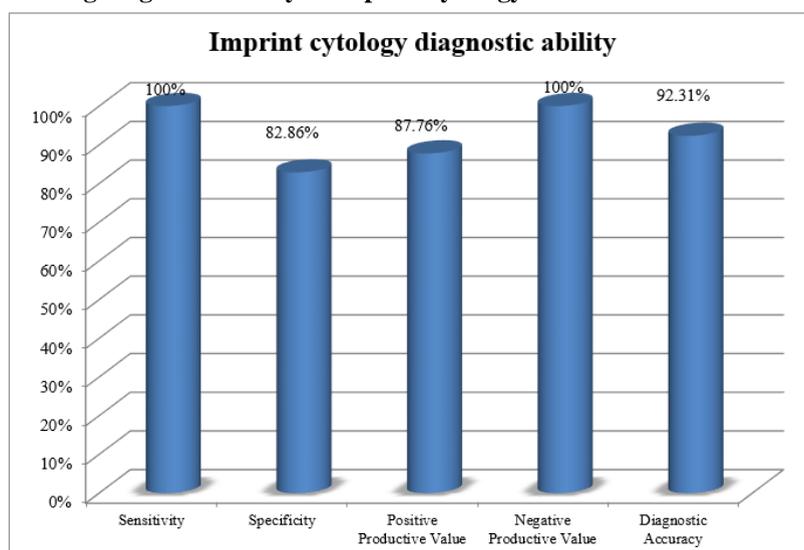
Graph 1: Age distribution of subjects



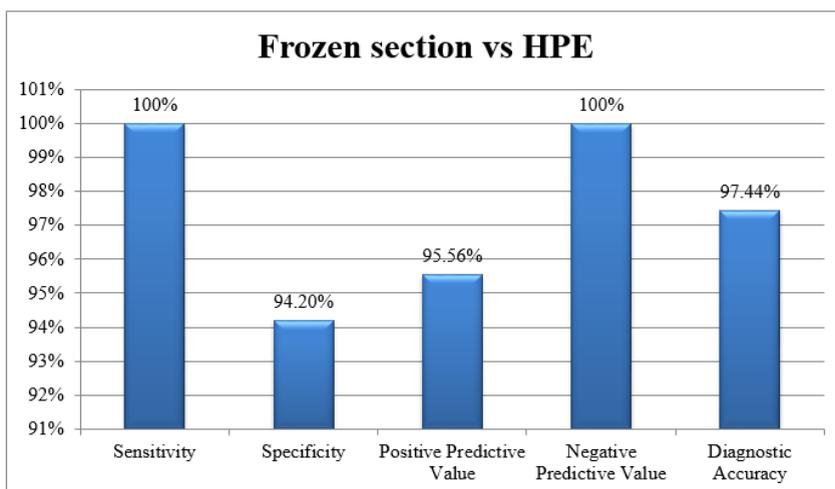
Graph 2: Sex distribution of subjects



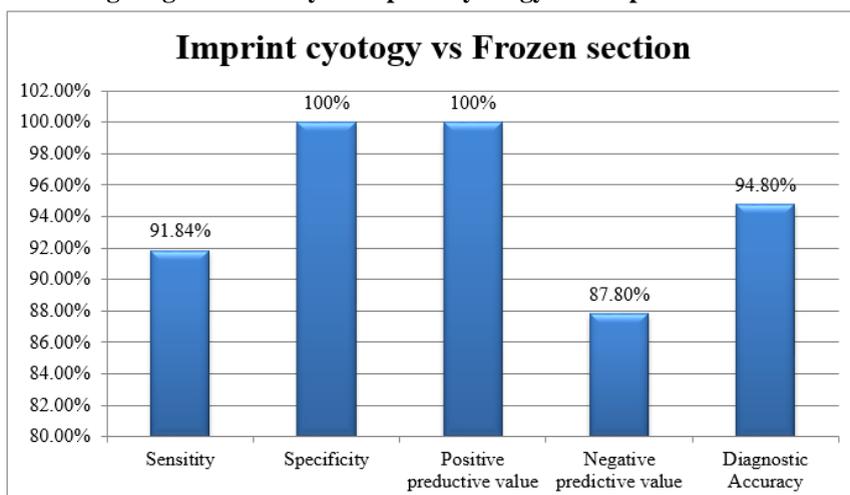
Graph 3: Bar diagram showing diagnostic ability of imprint cytology



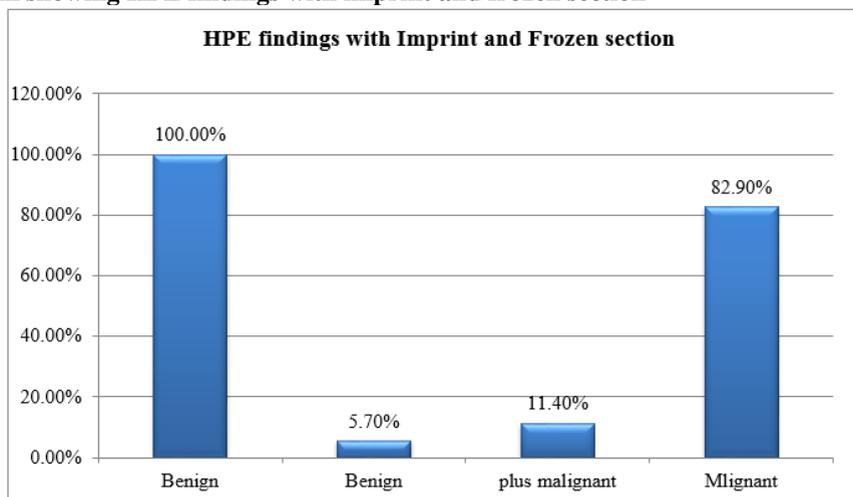
Graph 4: Bar diagram showing diagnostic ability of frozen section I breast lesions



Graph 5: Bar diagram showing diagnostic ability of imprint cytology in comparison with frozen section



Graph 6: Bar diagram showing HPE findings with imprint and frozen section



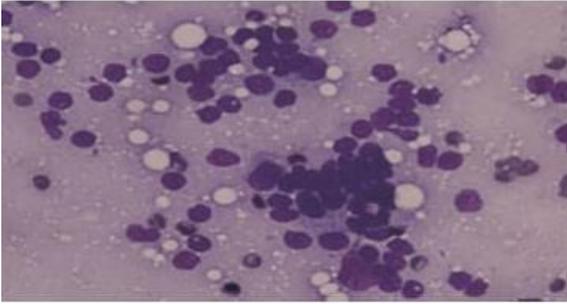


Fig. 1: Photomicrograph of touch imprint cytology of invasive ductal carcinoma showing discohesive cluster of pleomorphic ductal cells (Haematoxylin Stain X 20)

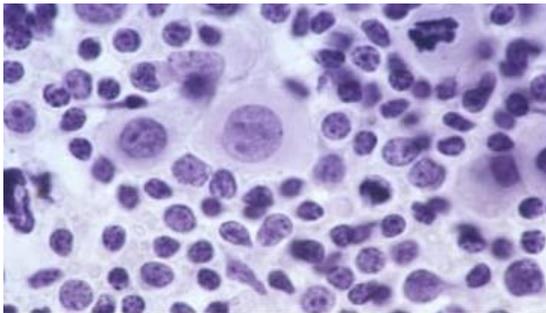


Fig. 2: Photomicrograph of FNAC smear showing cells with marked Pleomorphism, Prominent Nucleoli and Granular Chromatin (Papanicolaou 400)

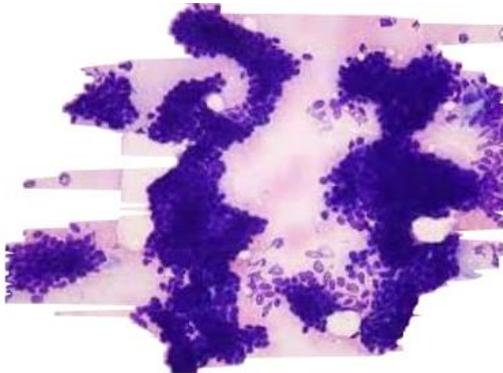


Fig. 3: Touch imprint cytology of phyllodes tumor of breast (Giemsa x 400)

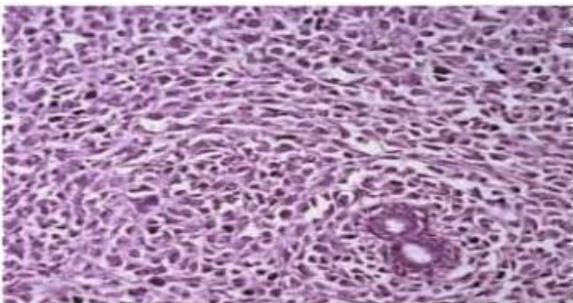


Fig. 4: Frozen section of phyllodes tumor of breast (H&E x 400)

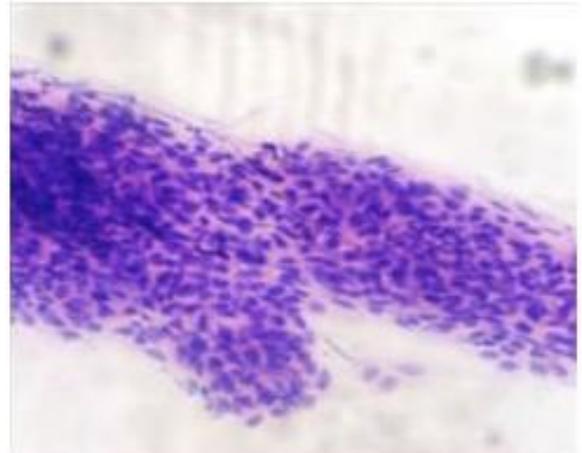


Fig. 5: Frozen section of breast papilloma (H&E x20)

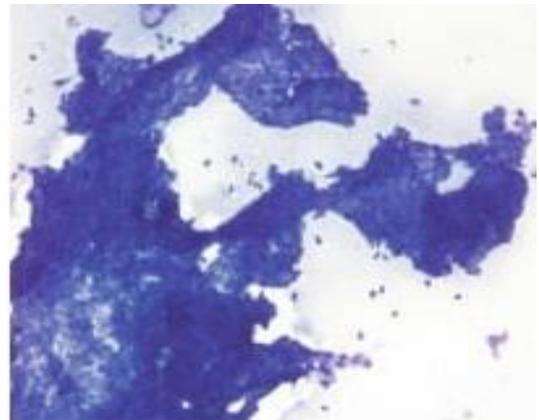


Fig. 6: TIC of breast papilloma (H&E x200)

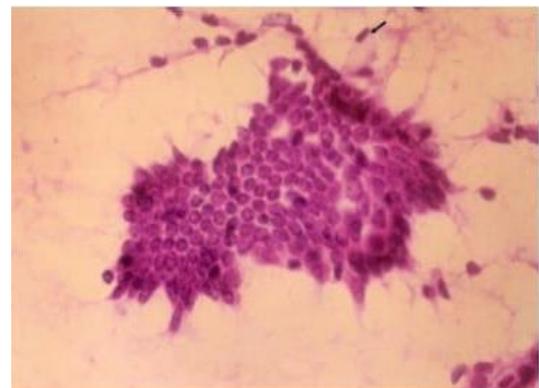


Fig. 7: TIC of the fibrocystic disease of the breast (H&E x20)

Conclusion

FS is superior to IC when compared with gold standard HPE in intraoperative diagnosis of breast mass lesions.

When the FS equipment is lacking, imprint cytology could be a reliable alternative with limited technical, financial provided that an experienced cytopathologist is available.

The predictive value analysis indicated that a positive diagnosis by IC is more reliable than a negative one.

IC can be used as an adjuvant to FS in the intraoperative consultations.

Conflict of Interest: None.

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