Evaluation of Accuracy of Intra Operative Imprint Cytology for Detection of Breast Lesions

Akhtar Z.M.,[1] Qureshi S.A.,[2] Aziz N.,[3] Niazi S.,[4] Qureshi S.S.,[5] Bukhari M.H.[6]
Address for Correspondence: *Department of Pathology, King Edward Medical University, Lahore*

Objective: To determine the accuracy of imprint cytology as an intraoperative diagnostic procedure for breast lesions with histopathological correlation.

Materials and Methods: This was a descriptive study on 40 cases of breast lesions comprising of inflammatory, benign & malignant lesions including their margins etc. It was conducted at King Edward Medical University, Lahore in collaboration with all Surgical Departments of Mayo Hospital. Relevant clinical data was recorded in a proforma. Both touch and scrape imprints were prepared from all the lesions and stained with May-Grunwaled Giemsa and Haematoxylin & Eosin stains. The imprints were subsequently compared with histopathology sections.

Results: When we used atypical cases as negative both touch and scrape imprints gave sensitivity, specificity, positive predictive value, negative predictive value and accuracy at 100%. However when we used cases with atypia as positive, sensitivity and negative predictive value were 100% with both touch and scrape imprints. Specificity, positive predictive value and accuracy were 71%, 86%, 85.5% respectively with touch imprints and 78%, 89%, 89% respectively with scrape imprints. No diagnostic difference was noted between the results of both stains. All the imprints were well correlated with histopathological diagnosis.

Conclusion: Imprint cytology is an accurate and simple intraoperative method for diagnosing breast lesions. It can provide the surgeons with information regarding immediate clinical and surgical interventions.

Keywords: Breast lesions, Fibroadenoma, Fibrocystic disease, Histopathology, Imprint Cytology, Malignant, Lobular carcinoma, Phylloides tumor.

Introduction

Lesions of the breast which are predominantly confined to the female, have gained a great deal of global attention because of increasing mortality and morbidity caused by breast cancer. Clinically, the majority of our patients presented with a palpable mass, inflammatory reddening, nipple discharge or mammographically detected abnormalities.[1,2] Majority of these conditions were clinically confused with malignancy. Data from the Western countries showed that about 70% of the women have fibrocystic disease (40%), fibroadenoma (7%), cancer (10%), inflammatory and others benign lesions (13%) while 30% of the patients had no pathological lesion in the breast.

In Pakistan the available data regarding the frequency of breast disease is different from that of Western countries because of lack of awareness, painless nature of the lump, socioeconomic and religious reasons. In Pakistan, carcinoma of the breast occurs in relatively younger age group, is more frequently seen in multiparous women and is of high grade associated with regional lymph node metastasis.[2,4-6]

Lesions of the breast including carcinoma are potentially curable, if diagnosed early and treated properly. For this purpose different strategies are in practice regarding the diagnosis and treatment of the breast lesions. Some of the diagnostic procedures are preoperative like mammography, ultrasound, FNA, core needle biopsy and incisional / excisional biopsy. Some diagnostic modalities are used intraoperatively like frozen section technique and imprint cytology. The diagnosis is ultimately confirmed by histopathological correlation.[7-14]

In the assessment of breast lesions, the most important role of diagnostic cytology is in making the binary decision between benign and malignant categories. Observation of normal and abnormal human cells, exfoliated or in imprints or scrapes was steadily and independently recorded throughout the nineteenth century.[15]

Procedures like FNA, core needle biopsy and imprints are revolutionized by the use of imaging modalities which enhances visualization and sampling of lesions that are not easily localized by older methods.[16]

Breast cytology is generally considered to be part of the initial assessment of breast lesions. It is also used as an adjunct to frozen section in paraffin section histology to assist in reaching a diagnosis.[17] Imprint cytology is a rapid and inexpensive diagnostic procedure which is being used for the diagnosis of lesions of various sites in the body.[12,13,14,18] This procedure has also been applied successfully on breast lesions. It is recommended to use both touch and scrape preparations to achieve better results.[10,17-20]

We used the criteria of Yiangou, et al (1996) for differentiating the benign and malignant breast lesions. The diagnostic classes were as C1–inadequate, C2- benign, C3-atypical probably benign, C4-suspicious probably malignant, C5-malignant.[11] We then correlated our cyto-

diagnosis with histopathological diagnosis, using modified Bloom Richardson grading system.[21] Lumpectomy margins were also evaluated by this method. Morphological aspects were taken into account while interpreting inflammatory and other breast lesions.

Materials and Methods

This study was conducted at King Edward Medical College, Lahore in collaboration with all surgical departments of Mayo Hospital. This was a descriptive study to assess the accuracy of imprint cytology in breast lesions completed in one year. The study was conducted on 40 breast lesions including neo-plastic, inflammatory, lumpectomy margins etc. Non probability convenience sampling. Patients included in this study were from female sex of post pubertal age group. It included all the lesions like neo-plastic, inflammatory, lumpectomy margins etc. Formalin fixed samples were excluded.

Data Collection Procedure

Relevant clinical data regarding age, history and clinical examination was recorded on a proforma. The lesions were identified and cut intraoperatively. Excessive haemmorhagic fluid was washed away. Gross examination of the lesions was recorded on the proforma. Four imprint smears were prepared from the lesion. Out of four, two imprints were obtained by gently pressing the clean dry glass slides against the cut surface of the lesion and rest of the two were scraped against the same site with the edge to get better cellularity. The material on the edge was smeared over the slide using another glass slide. The same area of lesion was submitted in 10% formalin. Slicing of the tissue was carried out for routine paraffin sectioning and staining with Heamatoxylin and Eosin. Both touch and scrape prepara-tions were stained with May-Grunwald Giemsa and Heamatoxylin and Eosin. The imprints were examined by panel of consultants and then the results were correlated with histopathological diagnosis. Findings of the imprints and histopathology were recorded on the proforma.

Table 1: *Cytological diagnosis of touch imprints of benign lesions.*

Diagnosis	No. of cases	C1	C2	C3	C4	C5
Fibroadenoma	6	0	3	3	0	0
Fibrocystic change	2	0	1	1	0	0
Benign phylloides tumor	1	0	1	0	0	0
Inflammatory	2	0	2	0	0	0
Lumpectomy margins	2	0	2	0	0	0
Infracted tubular adenoma	1	1	0	0	0	0
Total	14	1	9	4	0	0

Table 2: *Cytological diagnosis of scrape imprints of benign lesions.*

Diagnosis	No. of cases	C1	C2	C3	C4	C5
Fibroadenoma	6	0	4	2	0	0
Fibrocystic change	2	0	1	1	0	0
Benign Phylloides tumor	1	0	1	0	0	0
Inflammatory	2	0	2	0	0	0
Lumpectomy margins	2	0	2	0	0	0
Infracted tubular adenoma	1	1	0	0	0	0
Total	14	1	10	3	0	0

Table 3: *Cytological diagnosis of touch imprints of malignant lesions.*

Diagnosis	No. of cases	C1	C2	C3	C4	C5
IDC	24	0	0	0	1	23
Lobular carcinoma	1	0	0	0	1	0
Malignant phylloides tumor	1	0	0	0	1	0
Total	26	0	0	0	3	23

Table 4: *Cytological diagnosis of scrape imprints of Malignant lesions.*

Diagnosis	No. of cases	C1	C2	C3	C4	C5
IDC	24	0	0	0	0	24
Lobular Carcinoma	1	0	0	0	1	0
Malignant phylloides tumor	1	0	0	0	1	0
Total	26	0	0	0	2	24

Tissue Processing

An automatic tissue processor (Model RH-12 EP Sakura, Fine Technical Co. Ltd., Tokyo, Japan) was used for further processing of tissue blocks. About 17 hours were required for processing by this method. Different concentrations of ethyl alcohol were used for dehydration (70% alcohol for one hour x two changes, 90% alcohol for two hours x two changes and 100% alcohol for two hours x two changes). Clearing in two changes of xylene for one hour each. Tissues were impregnated in two changes of paraffin wax

with a melting point of 56°C for a period of 3 hours. Embedding of the tissues was done in paraffin wax using L-shaped metallic moulds. These blocks were put in the refrigerator for a period of 4-6 hours. Each block was cut on a rotatory microtome. About 3-4 micro meter thick tissue sections were obtained and placed in a water bath with a temperature of 5°C below the melting point of paraffin wax. Cut ribbons of tissues were placed on albumenized glass slides. 2-3 sections of the tissue were placed on each glass slide. All the sections and smears (both touched and scraped) were stained with haematoxylin and eosin.

Data Analysis

Screening tests were applied. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of imprint cytology were calculated taking histopathology as gold standard.

Results

There were fourteen benign and twenty six malignant lesions. Fibroadenomas was from younger age group. All were mobile, encapsulated and with well defined margins. The colour was grey white and surface was nodular. Histologically, mixed patterns were noted. Imprints revealed cohesive clusters of ductal epithelial cells with finger like projections. Many naked bipolar nuclei were seen. mild atypia was present.

Two cases of fibrocystic change were studied. The margins were ill defined. On cut section the lesions were leathery and whitish with cyst formation. Histologically, cystic changes, fibrosis and apocrine metaplasia were noted. Imprints revealed loose clusters of epithelial cells with many bipolar naked nuclei. Apocrine metaplasia and atypia were also noted.

One case of benign phylloides tumor was noted. This was firm in consistency. Microscopically, leaves like stromal nodules covered by epithelium projecting into the space were noted. Imprints revealed increased number of spindle shaped stromal cells.

Two cases of inflammatory lesions were noted adjacent to areola. Lesions were painful with overlying reddened skin. Cut section revealed dirty pink white oozing lesions. Margins were ill defined. Microscopically, acute on chronic mastitis and abscess formation were noted. Imprints revealed acute and chronic inflammatory cells.

Lumpectomy margins were identified due to tough whitish bands lining the cavity. Two cases were included. Histologically fibrosis was noted. Imprints revealed many macrophages against protienaceous background.

Table 5: *Touch and Scrape Imprints of Benign Lesions Considering Atypia as Negative.*

Diagnosis	No. negative	No. suspicious	No. positive
Fibroadenoma	6	0	0
Fibrocystic change	1	0	0
Benign Phylloides tumor	1	0	0
Lumpectomy margins	2	0	0
Inflammatory	2	0	0
Infracted tubular adenoma	1	0	0
Total	14	0	0

Table 6: *Touch Imprints of Malignant Lesions Considering Atypia as Negative.*

Diagnosis	No positive	No suspicious	No negative
IDC	23	1	0
Lobular Carcinoma	0	1	0
Malignant phylloides tumor	0	1	0
Total	23	3	0

Table 7: *Scrape Imprints of Malignant Lesions Considering Atypia as Negative.*

Diagnosis	No. positive	No. suspicious	No negative
IDC	24	0	0
Lobular carcinoma	0	1	0
Malignant phylloides tumor	0	1	0
Total	24	2	0

Table 8: *Screening test considering atypia as negative (Touch and scrap imprint).*

Test Results	Diseased	Not Diseases
Test Positive	TP = 26	FP = 0
Test Negative	FN = 0	TN = 14
Sensitivity = 100%, Specificity = 100%, PPV = 100%, NPV = 100%, Accuracy = 100%		

Table 9: *Screening test considering atypia as positive (Touch and scrap imprint).*

Test Results	Diseased	Not Diseases
Test Positive	TP = 26	FP = 4
Test Negative	FN = 0	TN = 10
Sensitivity = 100%, Specificity = 71%, PPV = 86%, NPV = 100%, Accuracy = 85.5%		

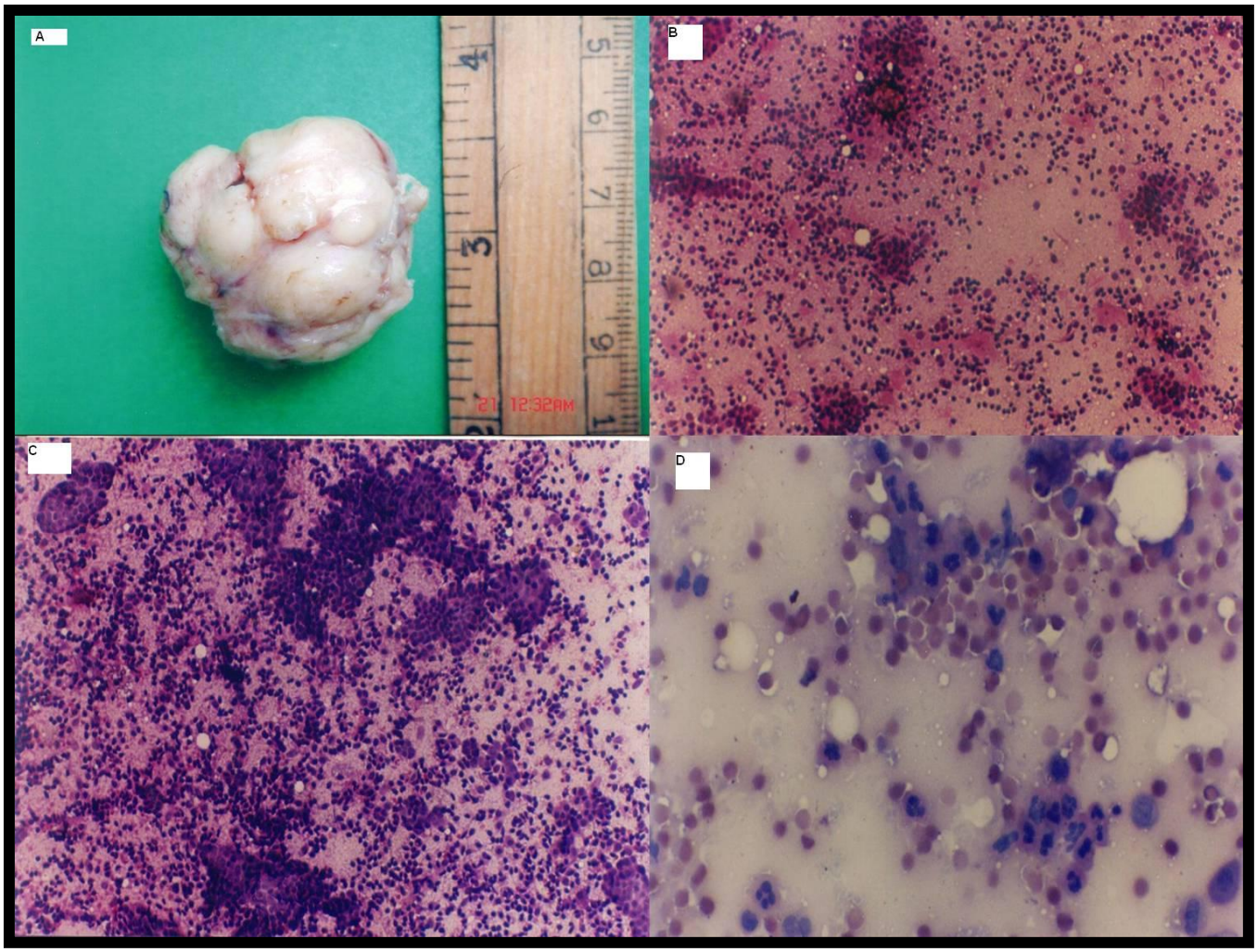

Fig. 1: *A-Gross picture Fibroadenoma after surgery, B. photomicrograph of touch imprint of Fibroadenoma (H&E 5x) showing dispersion of uniform cells among tight sheets of epithelial cells, C. photomicrograph scraping (H&E stain 5x) of tissue also shows same population of cells as seen in picture "C" but more crowding of cell population but stag horn pattern of clusters are more evident, D. photomicrograph acute mastitis (H&E stain 20x) on touch imprint*

One case of infracted tubular adenoma was included. It was encapsulated with red oozing cut surface. Histology revealed infracted tubular adenoma. Imprints revealed protienaceous background against which were seen many macrophages and nuclear debris.

We studied twenty six malignant cases. It included twenty four cases of invasive ductal carcinoma (no special type). After applying modified Bloom Richardson grading system, 18 cases were of grade III and six were of grade II. One case was of invasive lobular carcinoma and another was of malignant phylloides tumor. Imprints were well correlated with histological diagnosis. Indian file pattern was also appriciated in imprints helping diagnosis of lobular carcinoma. Features like pleomorphism, hyperchromatism and mitosis were clearly discernable on touch imprint as well.

All malignant cases were hard on palpation. No special drug history was noted. On cut section, all the lesions were hard with areas of fibrosis except lobular carcinoma which was rubbery in consistency. Cut surface was grey white in all cases.

Touch and scrape imprints were prepared from fourteen benign and twenty six malignant lesions. Cytological diagnosis was divided into five classes from C1 to C5. Smears were adequate in all the cases. In benign cases it was noted that no. of cases with atypia was more with touch imprints i.e, four while it remained three with scrape preparation. In malignant lesions, one case of IDC with C4 diagnosis on touch imprints turned out to be C5 on scrape preparation. Cytologic diagnosis remained the same with both H&E and May Grunwaled Geimsa stains. The cytological diagnosis was classified into three categories i.e.; negative, suspicious and positive. For practical purposes, cases with suspicious for malignancy were considered, as positive while

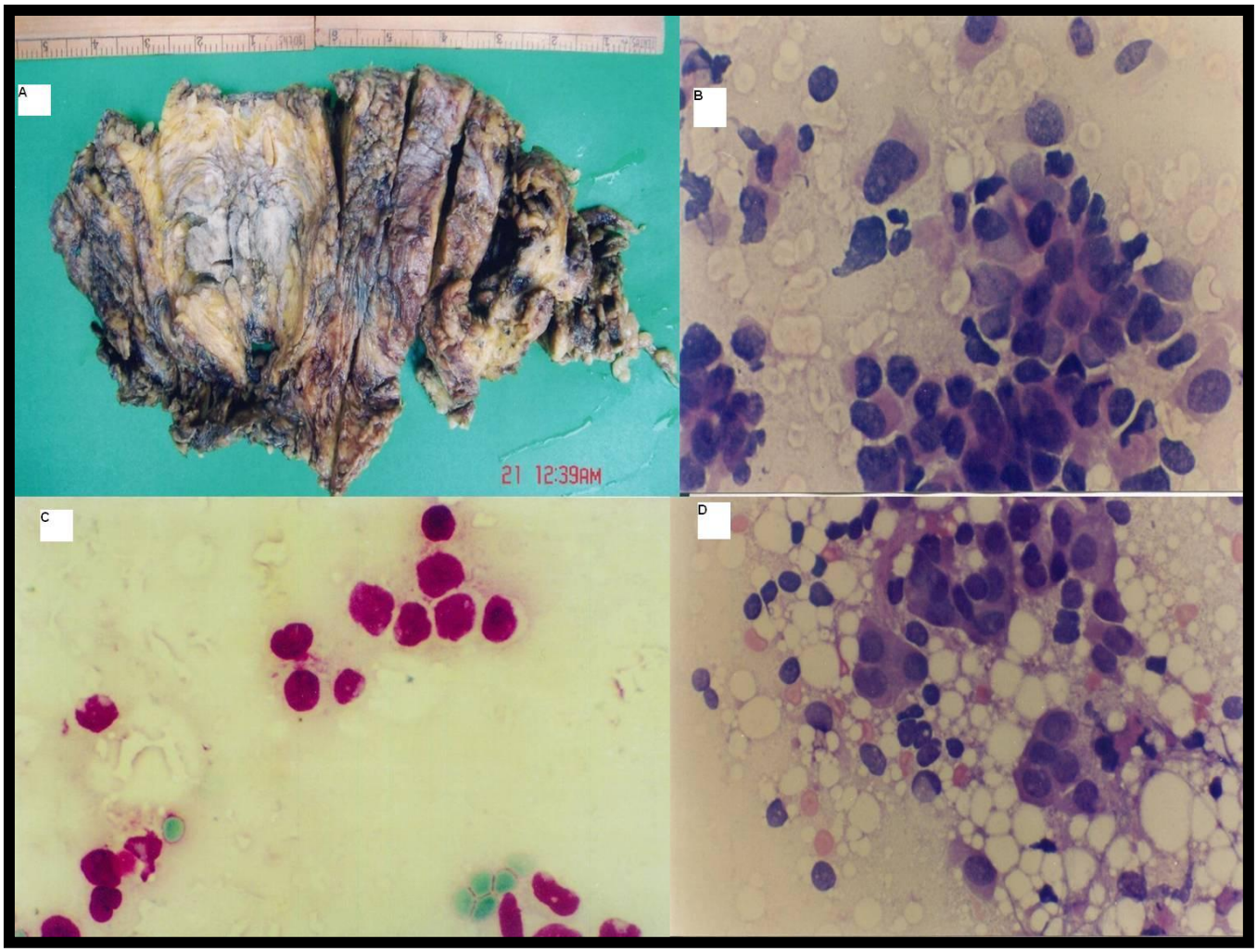

Fig. 2: *A-Gross picture Carcinoma Breast after surgery, B. photomicrograph of touch imprint of invasive ductal carcinoma (H&E stain 40x) showing pleomorphic and hyperchromatic cells and loose sheets of malignant epithelial cells, C. photomicrograph touch imprints (Giemsa stain 40x) malignant cells making Indian file pattern, D. photomicrograph fibrocystic disease, cells are showing apocrine changes but having regular nuclear membrane as compared to picture "C" (H&E stain 40x) on touch imprint.*

calculating the results by applying screening tests. (All the results are shown in Table 1-7 and photomicrographs Fig 1-2).

Discussion

The results of this study reveal that with satisfactory cellularity imprints of breast lesions are very accurate, with high sensitivity and specificity. In our study none of the malignancies were scored as benign and no benign lesions were reported malignant on imprint cytology. In benign cases it is noted that no. of cases with atypia are more with touch imprints i.e., four while it remains three with scrape preparations. In malignant lesions, one case of IDC with C4 diagnosis on touch imprints turns out to be C5 on scrape preparation and this was a case with grade II carcinoma. This gives advantage to scrape preparation over touch imprints. This result is well supported by the study conducted by Veneti S and co-workers who recommended scrape preparation to get better cellularity.[22]

When we consider atypia as negative (with touch and scrape imprints), sensitivity, specificity, PPV, NPV and accuracy remained 100%. No false positive or false negative cases are noticed. These results are very close to results by Veneti S and co workers where sensitivity, specificity and accuracy are 97.1%, 99.4% and 98.3% respectively.[22]

We noticed marked degree of differences in results when we consider atypia as positive cases. Here we find different results for touch & scrape preparation. Sensitivity and NPV remained 100% with both touch and scrape preparation however we find specificity, PPV and accuracy with scrape imprints as 78%, 89%, and 89% respectively. These results are different with touch imprints where specificity,

PPV and accuracy remains 71%, 86% and 85.5% respectively .This gives more edge to scrape preparation.

These results are particularly important in benign lesions. Cellular atypia is seen in some cases of fibroadenoma and fibrocystic change. These atypical findings are also described by Veneti et al and Jacobs et al.[22,23] Bipolar cells are helpful in differentiating these lesions from malignancies. Jacobs et al and Anlauf et al also recommended clinical correlation and complementary diagnostic procedures to avoid false diagnosis.[23,24] Jacobs T W and coworkers described the results of touch imprints of image directed breast CNB. Taking atypical and benign cases as negative, sensitivity and specificity were 94% and 95% respectively but when atypical cases were grouped with positive cases, sensitivity and specificity were 100% and 75% respectively.[23] These values are very near to our study results inspite of the fact that the Jacobs study was conducted with CNB which is a routine practice these days.

Dutta et al prefer scrape imprints of breast lesions with diagnostic accuracy of 94.1%. He included benign, malignant and inflammatory lesions in his study. His results also supports our results where accuracy was 100% considering atypical cases as negative. He recommended the technique as rapid intraoperative diagnostic procedure.[19]

In cases of evaluation of lumpectomy margins, the accuracy turned out to be 85% by Creager et al.[25] In our study we also successfully evaluated two such cases. This method allows survey of the entire surface area and allows reexcision to be performed during the initial surgery. Klimberg V S and associates also supported this method with sensitivity and specificity 100% respectively.[26] Cox CE and co-workers are also with similar views.[27] However Saarela et al did not recommend imprints as a method of assessing lumpectomy margins for at least early breast cancer. They were of the view that histologic margins are misleading in predicting residual cancer in re-excision specimen. They recommended wide excision or mastectomy in the management of multifocal and nonpalpable tumor to minimize the risk of residual cancer.[28]

Lee observed that imprint cytology can provide information about histologic patterns of the tumors but it can not provide information about the depth of infiltration.[29] We also noticed Indian file pattern in case of lobular carcinoma but at the same time we are also in favour of the second conclusion by Lee.[29]

We recommend the use of CNB for imprint cytology for evaluating breast lesions at out patient setting. It will be a same day procedure. However March et al did not recommend for immediate counseling and treatment planning[30] but we think that at least in benign lesions it can reduce patient anxiety. Like us, Veneti S et al also recommend this procedure because it can provide the surgeon with information regarding immediate clinical management.[22]

References

1. Lester SC. The breast. In: Kumar V, Abbas A K, Fausto N. Robbins and Cotran Pathologic basis of disease.7th ed. Philadelphia: Saunders 2004; 1119-54.
2. Lakhana NK, Khalid F. Morphology of breast lumps: a retrospective study. J Pak Inst Med Sci 2002; 13: 631-34.
3. Ellis H, Cox PJ. Breast problems in 1000 consecutive referrals to surgical out patients. Postgrad Med J 1984; 60: 653.
4. Malik IA. Clinico-pathological features of breast cancer in Pakistan. J Pak Med Assoc 2002; 52: 100-03.
5. Malik IA, Mushtaq S, Khan AH , Mamoon N, Afzal S, Jamal S, et al. A morphological study of 280 mastectomy specimens of breast carcinoma. Pak J Pathol 1994; 5: 5-8.
6. Qureshi JN, Qureshi ZA, Ahuja P, Memon AS Evaluation of breast carcinoma in breast lump. Pak J Surg 2001; 6: 7-9.
7. Mansoor I, Zahrani I. Analysis of inconclusive breast FNA by triple test. J Pak Med Assoc 2002; 52: 25-28.
8. Bedard YC, Pollet AF. Breast fine needle aspiration. Am J Clin Pathol 1999; 111: 523-27.
9. Scheiden R, Sand J, Tanous AM, Knolle U, Capesius C, Wagnon M CH, et al . Accuracy of frozen section diagnosis of breast lesions after introduction of a national programme in mammographic screening. Histopathology 2001; 39: 74-84.
10. Oneson RH, Minke AJ, Silverberg SG. Intraoperative pathologic consultation. Am J Surg Pathol 1989; 13: 237-43.
11. Yiangou C, Davis J, Liuni N, Barrett NK, Sinnett HD. Diagnostic role of cytology in screen detected breast cancer. Br J Surg 1996; 83: 816-19.
12. Rathi SL, Alam SM, Jamal Q. Touch imprints cytology of lymph nodes. J Coll Physicians Surg Pak 1997; 7: 203-04.
13. Shidham VB , Asma Z, Rao NR, Chavan A, Machhi J, Almagro U, et al. Intraoperative cytology increases the diagnostic accuracy of frozen sections of various tissues in the parathyroid region. Am J Clin Pathol 2002; 118: 895-902.
14. Ademiluyi SA, Akinyanju OO, Mordi VPN. Evaluation of lymph node imprints in rapid diagnosis of lymph node biopsy specimens. J Clin Pathol 1986; 39: 688-89.
15. Wied GC. Clinical Cytology: Past, present and future. Beitr Oncol 1990; 38: 1-58.
16. Naylor B, Ramzy I. Cytopathology: The past, the present and a glimpse into the new millennium. In: Gray W, Mckee GT. Diagnostic cytopathology. 2nd ed. Philadelphia: Churchill Livingstone 2003; 3-13.
17. Howat AJ, Williams RA. Combined scrape cytology and frozen section histology for rapid diagnosis in breast pathology. Histopathology 1990; 17: 85-96.
18. Helin HJ, Isola JJ, Helin MJ, Krohn KJE. Imprint cytology in: immunocytochemical analysis of oestrogen and progesterone receptors of breast carcinoma. J Clin Pathol 1989; 42: 1043-45.

19. Dutta SK, Chattopadhyaya A, Roy S. Evaluation of fine needle aspiration and imprint cytology in the early diagnosis of breast lesions with histopathological correlation. J Indian Med Assoc 2001; 99: 421-23.
20. Albert US, Duda V, Hadji P, Goerke K, Hild F, Bock K, et al. Imprint cytology of core needle biopsy specimens breast lesions . Acta Cytol 2000; 44: 57-62.
21. Dunne B, Going JJ. Scoring nuclear pleomorphism in breast cancer. Histopathology 2001; 39: 259-65.
22. Ventis S, Mouzaka LI, Toufeni H, Xemtides J, Anastasiadis P. Imprint cytology, a rapid, reliable method of diagnosing breast malignancy. Acta Cytol 1996; 40: 649-52.
23. Jacobs TW, Silverman IF, Schroeder B, Raza S, Baum JK, Schnitt SJ. Accuracy of touch imprint cytology of image directed breast core needle biopsies. Acta Cytol 1999; 43: 169-74.
24. Anlauf M, Nicklaus S, Rode G, Jackel C, Neumann K, Kalbfleisch H et al. Clinical experience with percutaneus large- core needle biopsies of the breast and evaluation of cytopathological and histopathological results. Zentralbl Gynakol 2003; 125: 353-61.
25. Creager AJ, Shaw JA, Young PR, Geisinger KR. Intraoperative evaluation of lumpectomy margins by imprint cytology with histologic correlation: a community hospital experience. Arch Pathol Lab Med 2002; 126: 846-48.
26. Klimberg VS, Westbrook KC, Korourian S. Use of touch preps for diagnosis and evaluation of surgical margins in breast cancer. Ann Surg Oncol 1998; 5: 220-26.
27. Cox CE, Pendas S, Ku NN, Reintgen DS, Greenberg JS, Nicosia SV. Local recurrence of breast cancer after cytological evaluation of lumpectomy margins. Am Surg 1998; 533-38.
28. Saarela AO, Paloneva TK, Rissanen TJ, Kiviniemi HO. Determinants of positive histologic margins and residual tumor after lumpectomy for early breast cancer: a prospective study with special reference to touch preparation cytology. J Surg Oncol 1997; 66: 248-53.
29. Lee TK. The value of imprint cytology in tumor diagnosis: a retrospective study of 522 cases in northern China. Acta Cytol 1982; 26: 169-71.
30. March DE, Walker MT, Bur M, Coughlin BF, Dziure B, Corenzane RR et al. Touch-preparation cytologic examination of breast core biopsy specimens: accuracy in predicting benign or malignant core histologic results. Acad Radiol 1999; 6: 333-38.