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COMPARATIVE STUDY OF IMPRINT CYTOLOGY AND HISTOPATHOLOGY IN DIFFERENTIATING BENIGN AND MALIGNANT TUMOURS OF THE BREAST IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Introduction:

Globally, breast cancer is the most common cancer among women; however, it is also a significant growing burden on the health systems of developing countries. Histopathology remains the gold standard for diagnosing all malignancies, including breast cancers. However, it is not a useful intraoperative diagnostic tool. Imprint cytology is a rapid as well as an inexpensive way to obtain a definitive diagnosis using minimal laboratory facilities. This study will compare the diagnostic accuracy of imprint cytology to histopathology in determining whether breast tumours are benign or malignant.

Materials and Methods:

This study was a hospital-based prospective comparative study performed at the Department of Pathology, ACS Medical College and Hospital, Chennai, India over a period of twelve months (May 2025 – April 2026). A total of thirty surgically excised breast specimens that met the study's entry criteria were enrolled in this study. The histopathological diagnoses were used as the gold standard to determine the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), overall diagnostic accuracy, and kappa coefficient of imprint cytology.

Results:

For the thirty specimens, there were a total of nineteen benign (benign breast disease) specimens and eleven malignant (untreated breast cancer) specimens. Imprint cytology had a sensitivity of 90.9%, a specificity of 100.0%, a PPV of 100.0%, an NPV of 95.0% and an overall diagnostic

accuracy of 96.7%. The kappa value (0.91) indicates that there was nearly perfect agreement between the two methods.

Conclusion:

Imprint cytology had excellent accuracy in distinguishing benign from malignant breast tumours. Invasive lobular carcinoma was the most difficult diagnosis to make. It was concluded that imprint cytology should be routinely used as an adjunct to histopathology to make intraoperative diagnoses, especially where cryostat facilities are unavailable.

Keywords: Imprint cytology; breast neoplasm; diagnostic accuracy; sensitivity.

INTRODUCTION

Breast Cancer is the most often diagnosed cancer in women worldwide, representing roughly 12.5% of all new cancer diagnoses in 2020 according to GLOBOCAN, with approximately 2.3 million new cases and 685,000 deaths that year.[1]

According to data from the National Cancer Registry Programme, breast cancer represents 27.7% of all females' cancers in metropolitan India, with Chennai, Mumbai and Delhi having the highest rates of incidence.[2] Among the more concerning aspects of breast cancer in India is the significantly younger mean age at diagnosis, and the number of breast cancers presenting at under 50 years of age is remarkably high; representing a significant proportion of cases.[3]

Timely and accurate assessment of whether a breast lesion is malignant or benign is central to clinical decision-making. The definitive diagnosis of breast cancer is made by histopathology on formalin- fixed paraffin-embedded tissue, allowing assessment of malignancy by evaluation of invasive properties, permitting tumour grading and allowing for the use of ancillary immunohistochemical stains.[4]

The principal drawback of conventional histopathology in the surgical setting is its inherent latency. Standard tissue processing requires a minimum of 24 to 48 hours.[5] Frozen section analysis partially addresses this limitation by providing an intraoperative result within 20 to 30 minutes; however, its implementation requires a cryostat machine, a skilled cryotomist, and a reliable cold-chain infrastructureresources that are not uniformly available across India.[6]

Imprint cytology, also termed touch imprint or contact smear cytology, involves pressing a clean glass slide gently but firmly onto the freshly cut surface of a surgically excised specimen, thereby transferring a monolayer of exfoliated cells that can be stained and examined under light microscopy within minutes.[7]

Sharma et. al evaluated some breast specimens with imprint cytology, compared these results with results from histopathology and found that the amount of cellularity on fresh surgical smears allowed for accurate diagnoses. The authors concluded that imprint cytology should be used as an additional diagnostic tool to frozen section analysis and may provide a reliable means of establishing an accurate diagnosis in resource-limited environments[8]. Mahadevappa et. al demonstrated that the combination of imprint cytology and frozen section provides improved intra-operative diagnostic accuracy when compared to the use of either device alone; therefore, both modalities should be used together whenever practical[9].

Devojee et. al used touch imprint cytology to evaluate 100 displaced breast specimens in comparison to histology and reported that there was a very good level of agreement across three major diagnostic categories; there was only one malignant case (an invasive lobular carcinoma, which is a well- established cancer type with cytologically subtle characteristics) that gave a dissimilar diagnosis from the two studies[10]. Ramraje et. al published an extensive comparative analysis that included a vast number of breast lesions and cited an overall sensitivity and specificity of 88% and >95% respectively with imprint cytology; false negative results were noted primarily in well-differentiated lobular carcinoma.[11]

Sauer et. al conducted touch preparation cytology on 966 breast core biopsies and reported sensitivities and specificities of 91% and 97%, respectively. Bofin et. al conducted imprint cytology

on excised specimens collected from 368 patients in Norway and reported similar results to those of Sauer et. al.[12]Klimstra et. al also reported that intra-operative cytologic examination can help establish surgical margins and identify sentinel lymph nodes in which rapid diagnosis can directly impact the surgical procedures performed[13].

The present study is aimed to prospectively compare imprint cytology with histopathology across a cohort of surgically excised breast specimens, calculating sensitivity, specificity, concordance, and kappa coefficient, and to identify specific lesion types that pose diagnostic challenges on imprint smears.

AIMS AND OBJECTIVES

To compare the diagnostic accuracy of imprint cytology and histopathology in differentiating benign and malignant breast tumours in a tertiary care hospital setting.

Objectives

1. To evaluate the sensitivity and specificity of imprint cytology in the diagnosis of breast tumours.
2. To compare cytological features observed in imprint smears with corresponding histopathological findings.
3. To assess the concordance rate between imprint cytology and histopathology in differentiating benign from malignant breast lesions.
4. To determine the utility of imprint cytology as a rapid intraoperative diagnostic tool for breast tumours.

MATERIALS AND METHODS

Study Design and Setting:

This was a hospital-based prospective comparative study conducted at the Department of Pathology, ACS Medical College and Hospital, Chennai, India. Institutional Ethics Committee approval was obtained prior to commencement, and the study was conducted in full accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants before enrolment.

Study Duration and Sample Size

The study was conducted over a twelve-month period from May 2025 to April 2026. Sample size was estimated based on a projected imprint cytology sensitivity of 90% with a 95% confidence level and an absolute precision of 10%, yielding a minimum requirement of 30 cases.

Selection Criteria

Inclusion criteria: All patients undergoing surgical excision of a palpable breast lump at the study institution were considered for enrolment, provided that adequate fresh tissue was available for both imprint smear preparation and subsequent histopathological processing.

Exclusion criteria: Cases were excluded if tissue samples were inadequate or poorly preserved, if the patient had a prior history of breast surgery, chemotherapy, or radiotherapy to the same breast, or if technical limitations—such as excessively fibrotic or necrotic tissue—precluded satisfactory smear preparation.

Imprint Cytology Technique

The pathology laboratory received freshly removed surgical specimens from operating rooms immediately following their removal (prior to chemical fixation). The grossly inspected specimens were serially sectioned at 1 cm intervals until the most representative portion of the lesion was identified. A clean labelled glass slide was then held against the surface of the freshly cut tissue for 2-3 seconds to obtain a cellular imprint. At least 2 smears were made for each case, ethanol-fixed

glass slides were processed with Haematoxylin & Eosin or Papanicolao stain. All imprints were reviewed by a pathologist, each case was classified as either benign, suspicious or malignant by applying pre- established criteria based on cytomorphologic appearance.

Histopathological Processing

Following imprint preparation, the remainder of the specimen was fixed in 10% neutral buffered formalin for a minimum of 12 hours. Tissue was processed through ascending grades of isopropyl alcohol, cleared in xylene, and embedded in paraffin wax. Sections of 3–5 microns were cut using a rotary microtome and stained with H&E. Special stains (Periodic acid–Schiff, Alcian blue) and immunohistochemistry (oestrogen receptor, progesterone receptor, HER2/neu) were performed where clinically indicated. The histopathological diagnosis served as the reference standard for all comparative analyses.

Statistical Analysis

Data were entered into Microsoft Excel and analysed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Imprint cytology diagnoses were cross-tabulated against histopathological diagnoses in a two-by-two contingency table. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy were calculated, with 95% confidence intervals estimated by the Wilson score method. The Cohen's kappa coefficient (κ) was used to quantify inter-method agreement and interpreted as: poor (< 0.20), fair (0.21–0.40), moderate (0.41– 0.60), substantial (0.61–0.80), or almost perfect (> 0.80). A p-value below 0.05 was considered statistically significant.

RESULTS

Thirty patients fulfilling the selection criteria were enrolled during the study period. All patients were female. The mean age of patients in the benign group was 32.4 ± 8.7 years (range: 18–54 years), compared to 49.1 ± 10.3 years in the malignant group, a difference that was statistically significant (p
< 0.001). Post-menopausal status was more prevalent among patients with malignant diagnoses (54.5%) than those with benign lesions (10.5%). Demographic characteristics are summarised in Table 1.

Table 1: Demographic profile of the study population by histopathological diagnosis

Characteristic	Benign (n = 19)	Malignant (n = 11)
Mean age ± SD (years)	32.4 ± 8.7	49.1 ± 10.3
Age range (years)	18 – 54	35 – 68
Right breast, n (%)	11 (57.9%)	6 (54.5%)
Left breast, n (%)	8 (42.1%)	5 (45.5%)
Pre-menopausal, n (%)	17 (89.5%)	5 (45.5%)
Post-menopausal, n (%)	2 (10.5%)	6 (54.5%)

Of the 30 specimens examined, 19 (63.3%) were classified as benign and 11 (36.7%) as malignant on histopathological examination. Among benign lesions, fibroadenoma was the most frequent diagnosis (n = 12; 40.0%), followed by benign phyllodes tumour (n = 4; 13.3%), fibrocystic change (n = 2; 6.7%), and breast abscess (n = 1; 3.3%). Among malignant lesions, invasive ductal carcinoma – Not Otherwise Specified (IDC-NOS) predominated (n = 7; 23.3%), followed by invasive lobular carcinoma (n = 2; 6.7%), medullary carcinoma (n = 1; 3.3%), and mucinous carcinoma (n = 1; 3.3%). The complete distribution is presented in Table 2.

Table 2: Distribution of histopathological diagnoses (n = 30)

Histopathological Diagnosis	No. of Cases	Percentage (%)

Benign Lesions (Total)	**19**	**63.3%**
Fibroadenoma	12	40.0%
Phyllodes tumour (benign)	4	13.3%

Fibrocystic change	2	6.7%
Breast abscess	1	3.3%
Malignant Lesions (Total)	**11**	**36.7%**
Invasive ductal carcinoma – NOS	7	23.3%
Invasive lobular carcinoma	2	6.7%
Medullary carcinoma	1	3.3%
Mucinous carcinoma	1	3.3%
Total	**30**	**100%**

Overall concordance between imprint cytology and histopathology was achieved in 28 of 30 cases (93.3%). Complete concordance (100%) was observed for fibroadenoma, fibrocystic change, breast abscess, IDC-NOS, medullary carcinoma, and mucinous carcinoma. Two discordant cases were identified: one case of benign phyllodes tumour that was misinterpreted as fibroadenoma on cytology due to the paucity of stromal elements in the imprint smear, and one case of invasive lobular carcinoma that was reported as a benign epithelial lesion owing to the deceptively bland cytomorphology of lobular carcinoma cells. Concordance rates stratified by diagnostic category are presented in Table 3.

Table 3: Concordance between imprint cytology and histopathology by diagnostic category

Diagnosis	Total	Concordant	Discordant	Rate (%)
Fibroadenoma	12	12	0	100%
Phyllodes tumour	4	3	1	75.0%
Fibrocystic change	2	2	0	100%
Breast abscess	1	1	0	100%
IDC – NOS	7	7	0	100%
Invasive lobular carcinoma	2	1	1	50.0%
Medullary carcinoma	1	1	0	100%
Mucinous carcinoma	1	1	0	100%
Total	**30**	**28**	**2**	**93.3%**

Using histopathology as the reference standard, imprint cytology yielded 10 true-positive, 19 true-negative, 1 false-negative, and 0 false-positive results (Table 4). The calculated diagnostic performance parameters are detailed in Table 5. Sensitivity was 90.9% (95% CI: 58.7–99.8%), specificity was 100.0% (95% CI: 82.4–100.0%), PPV was 100.0%, NPV was 95.0% (95% CI: 75.1–99.9%), and overall diagnostic accuracy was 96.7% (95% CI: 82.8–99.9%). The kappa coefficient was 0.91, indicating almost perfect inter-method agreement ($p < 0.001$).

Table 4: Two-by-two contingency table — imprint cytology vs. histopathological examination

	HPE: Malignant	HPE: Benign	Total
IC: Malignant	10 (TP)	0 (FP)	10
IC: Benign	1 (FN)	19 (TN)	20
Total	**11**	**19**	**30**

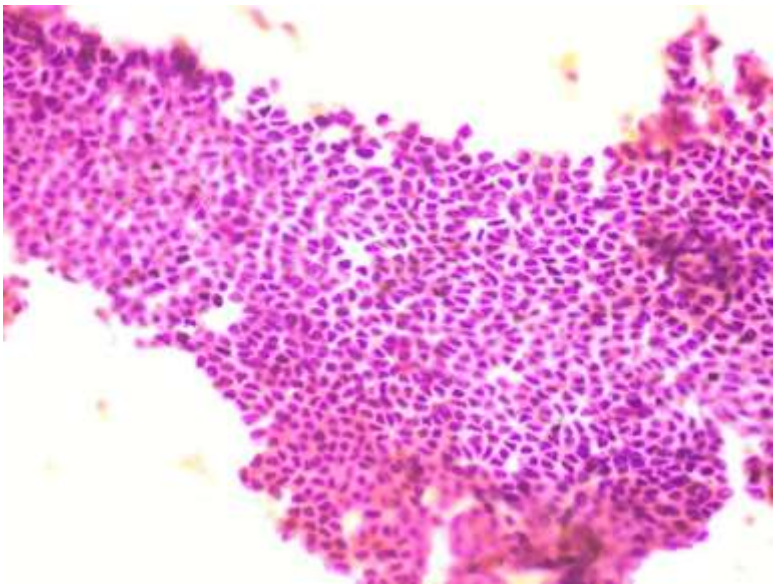
IC = Imprint Cytology; HPE = Histopathological Examination; TP = True Positive; TN = True Negative; FP = False Positive; FN = False Negative

Table 5: Diagnostic accuracy parameters of imprint cytology (n = 30)

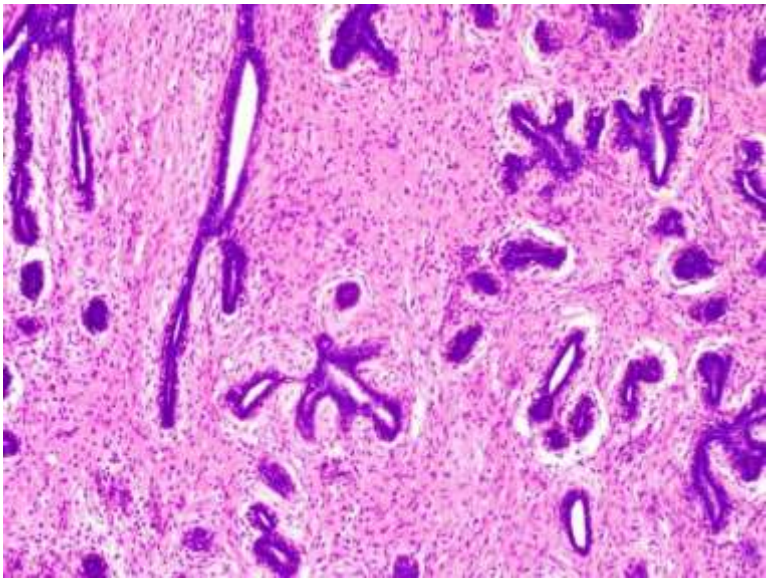
Diagnostic Parameter	Value (95% CI)
Sensitivity	90.9% (58.7 – 99.8%)
Specificity	100.0% (82.4 – 100.0%)
Positive Predictive Value (PPV)	100.0%
Negative Predictive Value (NPV)	95.0% (75.1 – 99.9%)
Overall Diagnostic Accuracy	96.7% (82.8 – 99.9%)
Kappa coefficient (κ)	0.91 (almost perfect agreement)
False-negative rate	9.1%
False-positive rate	0.0%

Smears from fibroadenomas contained cohesive epithelial groups in staghorn or antler-horn shaped clusters; bare bipolar nuclei scattered around; and a clear background, supporting a benign diagnosis (12 cases). IDC-NOS smears (7 cases) exhibited definitive malignant characteristics, in that they contained high cellularity, significant pleomorphic nuclei; irregular nuclear contours; defined nucleoli; high nuclear/cytoplasmic ratios; and tumor diathesis. The only false-negative lobular carcinoma

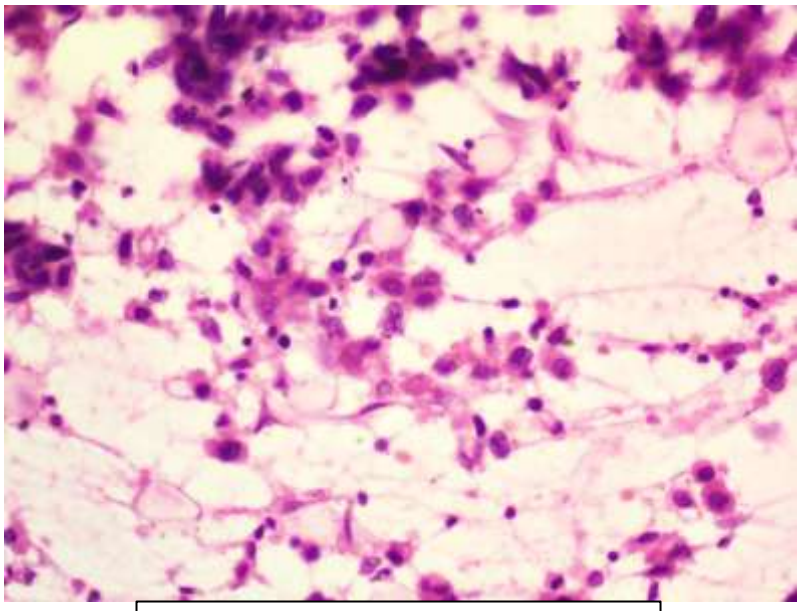
contained dispersed individual small cells, with mild nuclear enlargement, poorly-defined nucleoli; and minimal nuclear pleomorphism, resembling an epithelial proliferation that was benign in cytology. In the discordant phyllodes case, there was no representative stroma present on the imprint, therefore, the distinction could not be made between fibroadenomas and phyllodes based solely on cytology.



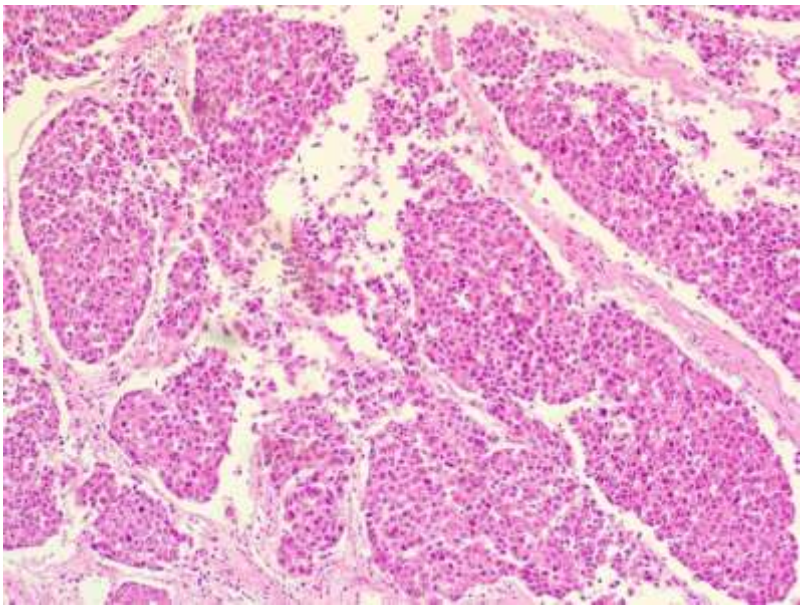
Imprint cytology (H&E) (10x view)
Fibroadenoma



Histopathology (H&E) (10x view)
Fibroadenoma



Imprint cytology (H&E) (10x view)
Invasive ductal carcinoma, NOS



Histopathology (H&E) (10x view)
Invasive ductal carcinoma, NOS

DISCUSSION

This clinical study assessed and compared the accuracy of imprint cytology to histopathology in 30 surgical excised breast specimens at a Tertiary care facility in Chennai, resulting in a 96.7% overall accuracy and kappa value of 0.91. Both values demonstrate almost perfect agreement between the two methods and are consistent with published literature from both Indian and international centers.[8-11]

Breast cancer, one of the top 8 cancers diagnosed in women globally, requires timely access to accurate information for properly determining treatment guidelines as the methodologies for treating the disease have become extremely complicated.[1,2]

The 100% concordance observed for IDC-NOS in this series aligns with findings reported by Devojee et al., who recorded complete agreement between touch imprint results and histopathology for all ductal carcinoma cases in their 100-case prospective cohort.[10] This concordance is biologically expected: IDC-NOS cells exhibit pronounced cytological atypia, nuclear enlargement, coarse chromatin, prominent nucleoli, and loss of cohesiveness makes malignant identification on imprint smears straightforward.[14] Similarly, medullary and mucinous carcinomas, each represented by a single case in this study, showed 100% concordance, consistent with the recognisable cytomorphological signatures of these well-characterised special subtypes.[11]

The single false-negative diagnosis in this series was an invasive lobular carcinoma. This finding is neither surprising nor isolated: lobular carcinoma has been documented as the most consistently problematic subtype for cytological diagnosis across multiple published series, owing to its characteristic cellular dyscohesion, small cell size, low nuclear grade, and the absence of prominent nucleoli or mitoses.[10,11] Devojee et al. similarly encountered one false-negative among four lobular carcinoma cases in their cohort, and Ramraje et al. identified lobular carcinoma alongside well-differentiated ductal carcinoma as the principal sources of false-negative diagnoses.[10,11] Pathologists evaluating breast imprint smears should therefore maintain a low threshold for suspicion when encountering smears with individually dispersed small cells displaying only mild nuclear abnormality, even in the apparent absence of definitive malignant features.[13]

The discordant phyllodes tumour case, interpreted cytologically as fibroadenoma due to the absence of stromal fragments in the imprint, illustrates a second well-established limitation of touch imprint cytology: the inability to reliably distinguish phyllodes tumour from fibroadenoma when the characteristic leaf-like stromal component is not transferred to the smear.[15]Mahadevappa et al. encountered analogous difficulties with phyllodes tumours in their intraoperative.

The zero false-positive rate in this study deserves specific attention. No patient with a benign lesion received an erroneous malignant diagnosis on imprint cytology, yielding a PPV of 100%. This means that every patient reported as malignant on cytology was confirmed malignant on histopathology,[12] thus eliminating the risk of unnecessary radical surgery.

The kappa coefficient of 0.91 recorded in this study exceeds the 0.80 threshold conventionally used to define almost perfect agreement and compares favourably with values reported in comparable prospective series.[8,9] Sauer et al. reported a kappa of 0.88 for touch preparation cytology from breast biopsies in a Norwegian population, while Bofin et al. recorded a kappa of 0.85 for imprint cytology from excision specimensboth series using histopathology as the gold standard, as was the case in the present study.[12]

The practical advantages of imprint cytology is cost effective requiring only glass slides, a basic staining setup, and a functioning light microscope, all of which are standard equipment in any pathology laboratory.

The primary limitation of the present study is its relatively small sample size of 30 cases, a consequence of the single-centre design and the twelve-month study duration. The wide confidence intervals around the sensitivity estimate (58.7–99.8%) reflect this limitation and underscore the need for a larger multicentre study to generate more precise estimates.[16]

CONCLUSION

Imprint cytology demonstrated a diagnostic accuracy of 96.7% and a kappa coefficient of 0.91 in the evaluation of breast tumours when compared to histopathological examination as the gold

standard, confirming its status as a reliable, rapid, and cost-effective intraoperative diagnostic adjunct.Sensitivity was marginally reduced by the diagnostic challenge inherent to invasive lobular carcinoma, a recognised cytological pitfall, while specificity and PPV were both 100%, ensuring that no patient with a benign lesion received a false malignant label. Given its minimal infrastructure requirements and the acute need for affordable intraoperative diagnostic tools across India, imprint cytology should be considered for formal incorporation into the routine breast specimen assessment protocol at tertiary care and district-level institutions, particularly where frozen section facilities are unavailable.

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