

## Evaluation of Gray Zone Breast Lesions Along with Cyto-Histological Correlation

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### Abstract

**Background:** Breast cancer has recently surpassed cervical cancer as the most frequent malignancy in Indian women. In order to distinguish between benign and malignant breast lesions, FNAC is a very safe, minimally invasive, and quick diagnostic technique. The National Cancer Institute (NCI) has developed a thorough and consistent system in 1996 for classifying FNAC breast lesions into five groups for the diagnosis as follows: inadequate - C1, benign - C2, atypical, probably benign - C3, suspicious, favours malignancy - C4, and malignant - C5. C3 and C4 are gray lesions that are difficult to interpret for a specific diagnosis.

**Aims and Objectives:** The objective of this study is to evaluate the significance of FNAC in the C3 and C4 category diagnosis and to compare it to histopathological diagnosis.

**Materials and Methods:** A retrospective study was carried out in the Department of Pathology of Silchar Medical College and Hospital, from 1 September 2020 to September 2022. A total of 312 cases were studied, and those that fell into the C3 and C4 categories were selected for further investigation and later histopathological results were compared.

**Results:** Out of 312 cases which were studied, C3 and C4 categories constituted 18 (5.7%) and 24 (7.69%) cases respectively. Histopathological diagnosis was available in 9 cases of C3 (50%) and 16 cases of C4 (66.66%). Among C3 category, 7/9 cases in which histopathological examination was available showed benign lesions (77.77%) and 2/9 cases were malignant (22.22%). Among C4 category, 2/16 cases available for histopathological examination showed benign lesions (12.5%) and 14/16 cases turned out to be malignant (87.5%). Between the C3 and C4 categories, there was a statistically significant difference between benign and malignant lesions ( $p < 0.001$ ).

**Conclusion:** Thus, FNAC of breast is a simple, safe, quick, and affordable diagnostic modality that is crucial in the detection of grey zone breast lesions. However, it is important that clinicians understand the limitations of FNAC. There was a statistically significant difference in benign & malignant diagnosis for these categories in our study, and so C3 and C4 categories should still be continued with.

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### Introduction

Breast cancer has recently surpassed cervical cancer as the most frequent malignancy in Indian women. [1] According to data from ICMR, 1.5 lakh (10% of all malignancies) new cases were estimated to have been reported in 2016, making it the most common cancer overall. [2] It is one of the crucial organs that undergoes fine needle aspiration cytopathology (FNAC) on a regular basis for the identification of diseases, mainly malignancy. In order to distinguish between benign and malignant breast lesions, FNAC is a very safe, minimally invasive, and quick diagnostic technique. The utility of FNAC has also been

increased by the method's ease of use, relative affordability, and high sensitivity, which has been demonstrated to be in the range of 76-99%. [3] But there are some lesions that are referred to as grey lesions of the breast and whose diagnosis is challenging to understand. The National Cancer Institute (NCI), has developed a thorough and consistent system in 1996 for classifying FNAC breast lesions into five groups for the diagnosis as follows: inadequate - C1, benign - C2, atypical, probably benign - C3, suspicious, favours malignancy - C4, and malignant - C5. [4] Gray lesions that are difficult to interpret for a specific diagnosis include categories C3

and C4.[5] For such ambiguous C3 and C4 categories, some authors use the word "equivocal." This method has helped the surgeon prioritise patients for various treatment techniques. [6] As a first line diagnostic method, FNAC still has a lot to offer, especially in developing nations with limited resources. For the preoperative diagnosis of breast cancer, fine needle aspiration cytology (FNAC) is increasingly used. In a conference sponsored by Bethesda on breast cancer prevention, Tumor grading based on FNAC material was also advised to be also included in FNA reports for prognostication.[7] Structured reporting will help with patient management, improve breast health care, and make it easier for future study. Also, it can improve report quality, consistency, and reproducibility across divisions, cities, and countries. [8,9,10] For histologically evaluating breast cancer, the modified Scarff-Bloom-Richardson (SBR) grading system is widely used. Thus, grading of breast cancer should be included in FNAC data for prognostication in the era of neo adjuvant chemotherapy. [11]

### Aims and Objectives

The objective of this study is to evaluate the significance of FNAC in the C3 and C4 category diagnosis and to compare it to histopathological diagnosis.

### Materials and Methods

This study is a retrospective study carried out in the Department of Pathology of Silchar Medical College and Hospital, from 1 September 2020 to September 2022, after taking the necessary approval from the institution. In the present study, 312 cases were included, and those that fell into the C3 and C4 categories were selected for further investigation and later histopathological results were compared.

### Inclusion Criteria

Included all breast lesions with C3 and C4 criteria on cytology.

### Exclusion criteria

Included inconclusive fnac and other categories on fnac examination (C1, C2, C5). FNAC was done with 22-gauge 10 ml syringes. Aspirated material was stained with May-Grünwald/ Giemsa stain. Surgically removed breast tissues, including those from biopsies and mastectomies supplied in 10% formal saline, were fixed, grossed, and processed. Several sections were cut at a thickness of 3–4  $\mu$ , stained with H & E, and analysed. In cases where biopsy was performed after FNAC diagnosis, the cytological diagnosis and histological diagnosis were compared. The statistical significance of benign and malignant lesions in both these categories was calculated. For statistical significance, a p value of 0.05 or less was taken into consideration.

### Results

A total of 312 cases were studied. The patients ranged in age from 15 to 76 years. The most prevalent age range was between 21 to 30 years.[Table 1] Out of these, C3 and C4 categories constituted 18 (5.7%) and 24 (7.69%) cases respectively.[Table 2] All of the patients in these two categories were female. Among these categories, the age ranged from 28 – 62 years. Histopathological diagnosis was available in 9 cases of C3(50%) and 16 cases of C4 (66.66%).[Table 3] Infiltrating ductal carcinoma was the most frequent malignancy and in benign cases fibroadenoma was most commonly seen after histological diagnosis. Among C3 category, 7/9 cases in which histopathological examination was available showed benign lesions (77.77%) and 2/9 cases were malignant (22.22%).

Among C4 category, 2/16 cases available for histopathological examination showed benign lesions (12.5%) and 14/16 cases turned out to be malignant (87.5%).

Between the C3 and C4 categories, there was a statistically significant difference between benign and malignant lesions ( $p < 0.001$ ).[Table 4].

**Table 1: Age-wise distribution of patients with grey lesions**

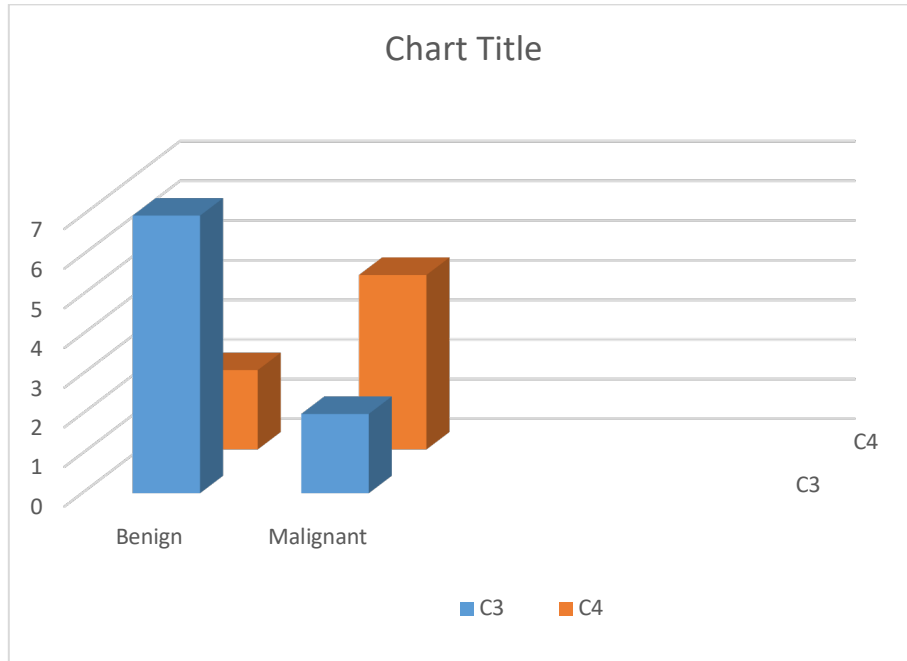
Age distribution	<21 years	21-30 years	31-40 years	41-50 years	51-60 years	61 and above	Total
Male	1	2	0	0	1	1	5 (1.6%)
Female	38	105	68	46	35	15	307 (98.39%)
Total (%)	39 (12.5%)	107(34.29%)	68(21.79%)	46(14.74%)	36(11.53%)	16(5.12%)	312

**Table 2: Fine-needle aspiration cytopathology distribution of patients in grey zone (C3 and C4)**

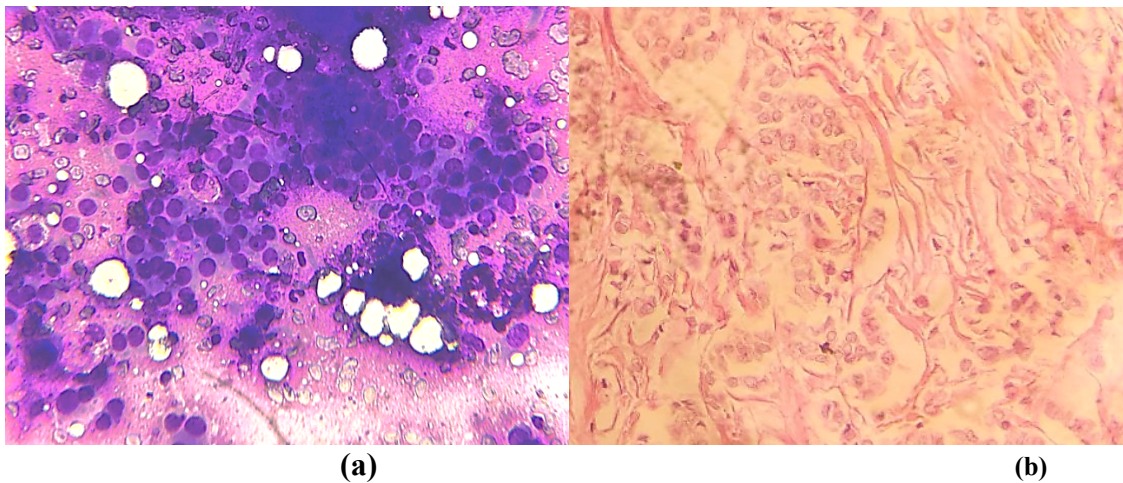
FNAC	C3	C4	Total
Fibroadenoma	04	02	06
Fibroadenoma with atypia	03	08	11
Proliferative breast disease	04	06	10
Fibrocystic disease	04	02	06
Phyllodes tumour	02	03	05
Papilloma	00	01	01
Gynecomastia	01	01	02
Apocrine adenoma	00	01	01
Total	18	24	42

**Table 3: Cytohistological correlation of C3 and C4 lesions**

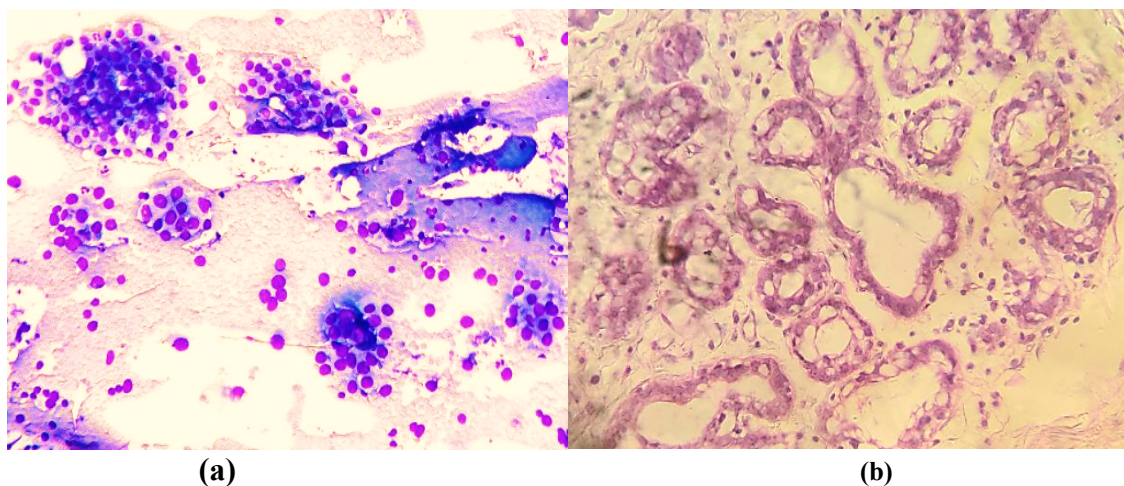
Histopathological Diagnosis Available	FNAC C3	FNAC C4	Total
Benign	7 (77.77%)	2 (12.5%)	09
Malignant	2 (22.22%)	14 (87.5%)	16
Total	9 (100%)	16 (100%)	25



**Figure 1: Bar diagram representing benign and malignant cases of C3 and C4 category**



**Figure 2: False-negative case – Atypical, probably benign (C3), turning out to be malignant. (a): FNAC category C3 showing clusters of ductal epithelial cells exhibiting mild discohesion and mild nuclear atypia along with few scattered myoepithelial cells. (MGG, 10 X 40), (b): Follow-up histopathological section showing invasive ductal carcinoma. (H & E, 10 X 40)**



**Figure 3: False-positive case – Suspicious, probably malignant (C4), turning out to be benign. (a): FNAC category C4 showing ductal epithelial cells exhibiting dyscohesion with moderate nuclear atypia. (MGG, 10 X 10), (b): Subsequent histopathological examination revealing features of fibroadenoma with glandular adenosis. (H & E, 10 X 40)**

### Discussion

Different breast lesions range from mastitis to malignancy.[12] The best methods for diagnosing breast lesions include clinical examination, ultrasonography, and FNAC. The distinction between benign and malignant lesions can be made using FNAC, a well-established diagnostic technique for the diagnosis of various breast lesions.[13]

The term "grey lesions" refers to breast lesions that are difficult to diagnose due to this differentiation. The National Cancer Institute has a few recommendations that fall under the following classifications: insufficient - C1, benign - C2, atypical - C3, suspected for malignancy - C4, and malignancy - C5. The pathologist faces difficulties with categories C3 and C4, as there are no set standards for diagnosis. [5,14,15].

An interpretation of C3 is provided when the aspirate demonstrates benign characteristics, but some features, such as cellular crowding, nuclear pleomorphism, loss of cell cohesion, nuclear-cytoplasmic changes, or therapy related nuclear-cytoplasmic changes resulting from treatment/hormonal changes which raise possibilities for malignancy. Whereas the C4 category is assigned when the morphology promotes the likelihood of malignancy, but microscopic examination reveals insufficient cellularity, spread, or preservation, and it may also be masked by bleeding or inflammation.[15] Also, it contains samples that exhibit characteristics that are more pronounced than those in C3 but lack cells that are obviously malignant.[5,13] The patients in our study were between 15 – 76 years of age. The maximum number of cases was in the age range between 21 – 30 years, i.e. almost similar to the study conducted by Madan et al. and Yusuf I et al.[4,13] For the above mentioned categories the age ranged from 28 – 62

years of age and is comparable to studies conducted by Arun P et al. and Shabb NS et al.[5,14]

C3 and C4 categories combined constituted 42/312 (13.46%) cases subjected to breast FNAC during the study period. This percentage of C3 and C4 categories correlated well with various other studies of Arul P et al, Shabb NS et al., Mitra S et al, Goyal P et al, Kanhough R et al, Howell LP et al, all of which give a range of 4-17.7% for both, these categories combined. [5,14,15,16,17,18] This is crucial because there shouldn't be an incorrect overuse of these categories when reporting breast FNAC. In C3 category, where 9/18 (50%) cases were available for histopathological examination, 2 cases turned out to be (IDC) infiltrating duct carcinomas (2/9= 22.22%) and thus were considered false negative (FN). This result also corroborated well with the range established by other studies Arul P et al, Shabb NS et al., Goyal P et al, Kanhough R et al, Deb RA et al, (8.6-52%). [5,14,16,17,19] These false negative cases may have occurred due to sampling error, low-grade tumours, small tumour sizes, low cellularity, or low-grade, well-differentiated carcinomas that formed in cystic lesions. These two FN cases were again reviewed after histopathological diagnosis. In each of these cases, the ductal epithelial cells primarily appeared as cohesive sheets with bare nuclei and few clusters demonstrating cellular crowding and lack of cohesion. [Figure 1] As a result, patients with a C3 diagnosis do not require surgery if the appropriate clinical and mammographic correlation is performed and both methods indicate a benign disease. In C4 category, 16/24 cases (66.66%) which were available for histopathological examination showed malignant pathology i.e. invasive ductal carcinoma (14 cases) and invasive lobular carcinoma (2 cases). 3/24 cases (12.5%) showed benign pathology on histopathological examination and were considered *false*



positive (FP). Of these 2 were highly cellular fibroadenomas and 1 was proliferative breast disease. These cases displayed mild cellular and nuclear pleomorphism, dyscohesive clusters, and cellular overlapping. [Figure 2] These results also correlate with the other studies, which show a range of 81-97% for malignancies in this category i.e., Arul P et al, Kanhough R et al, Deb RA et al., Lim JC et al, Yusuf I et al, Tikku G et al, Omi Y et al[5,17,19,20,13,21,22]. Atypia, dyscohesion, and nuclear pleomorphism, combined with enhanced cellularity, can be present in fibroadenomas to varying degrees and make a diagnosis challenging. The majority of these cases are conventional fibroadenomas, while a few of them may also include proliferative lesions, especially in the presence of atypical features. Another grey zone lesion that can be referred to as the "nightmare of the pathologists" is proliferative breast disease, especially radial scar and complex sclerosing lesions. These lesions can be hypercellular, have dyscohesive cell clusters, atypia, and absence of myoepithelial cells in few clusters. As a result, due to the extremely high prevalence of cancers in C4 lesions on breast FNAC, all patients with this diagnosis should undergo surgery.

### Conclusion

To summarise, FNAC of breast is a simple, safe, quick, and affordable diagnostic modality that is crucial in the detection of grey zone breast lesions, which can be challenging and confusing at times. However, it is important that clinicians understand the limitations of FNAC and histopathology examination must be done in these cases to rule out and exclude malignancy. While there was a statistically significant difference in benign & malignant diagnosis for these categories in our study, C3 and C4 categories should still be used. Lastly, cytology followed by histology, and further ancillary radiological investigations like mammography and ultrasonography can all help diminish grey zone lesions.

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