Cyto-Morphological Correlation of Indeterminate C3 (Atypical) and C4 (Suspicious) Categories in Fine Needle Aspiration Cytology of the Breast

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Abstract- In 1996, to harmonize the reporting system of breast lesions on fine-needle aspiration cytology (FNAC), National Cancer Institute (NCI) proposed five categories from C1-C5. C3 and C4 categories are noted to be ambiguous during histopathological correlation, hence "grey-zoned." There have been limited researches regarding its usefulness and its histopathological correlation. This study was undertaken to evaluate C3 and C4 categories and to correlate with histopathological examination. This perspective and retrospective study was undertaken for two years. Forty cases of C3 and 32 cases of C4 were retrieved from a total of 602 cases of breast FNAC. Histopathological follow-up and correlation were available in 30 cases of C3 and 15 cases of category C4 and were selected for further study. On the histopathological correlation of the C3 category, 22 (73.3%) cases turned out to be benign, and 8 (26.7%) cases revealed malignant diagnosis, and among the C4 category, 2 (13.3%) cases revealed benign findings, and 13(86.7%) of the cases showed malignancy. This difference was statistically significant (P<0.001). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the C4 category in the diagnosis of malignancy were 61.9%, 91.6%, 86.6%, and 73.3%, respectively. FNAC is a simple, rapid, cost-effective, and accurate method to diagnose easily accessible breast swellings. However, one should be aware of its limitations as well. Our study supports maintaining C3 and C4 categories, as there was a statistically significant difference in benign & malignant diagnosis for these categories

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Introduction

Breast cancer is the most common cancer among women worldwide, representing about 25% of all cancers. In India, there was recorded an increase of 11.54% in incidence and 13.82% in mortality of breast cancer patients during 2008-2012. These alarming figures are due to a lack of adequate breast cancer screening procedures and their detection at an advanced stage (1).

Fine needle aspiration cytology (FNAC) has always been an indispensable tool to diagnose easily accessible swellings, most notably breast. Despite the growing affection for advanced imaging modalities and core needle biopsies (CNB) in developed diagnostic centers, FNAC still holds promise at places where such techniques are unavailable. FNAC still plays an important role in the following areas- diagnosis of benign lesions in symptomatic palpable breast lumps as part of the triple assessment (clinical examination, mammography and, FNAC), the staging of breast carcinoma, specifically preoperative axillary lymph node FNAC and intraoperative sentinel node imprints, and detection of metastasis at distant places after treatment for primary tumor (2). However, FNAC has its drawback of insufficiency to differentiate between certain benign or indeterminate lesions from malignant lesions and subcategorize the lesions.

In 1996, to harmonize the reporting system of breast lesions on FNAC and to overcome its limitations,

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National Cancer Institute (NCI) categorized the findings into five groups, *i.e.*, C1-C5 (3). Categories C1 (inadequate), C2 (benign) and, C5 (malignant) pose a little problem in diagnosis and are straightforward. However, C3 (atypical, probably benign) and C4 (suspicious for malignancy) categories are noted to be ambiguous during histopathological correlation, hence "grey-zoned." Howell (4) and Kanhoush *et al*., (5) coined the term "equivocal" in their studies to describe indeterminate C3 and C4 categories on breast FNAC. The "grey zone" (C3 and C4) constitutes around 8.9% (6) and ideally should not exceed 15% of all FNA diagnoses to prevent overuse and abuse of such ambiguous categories (7).

Since the advent of the above-mentioned classification system, there have been limited researches regarding its usefulness, its histopathological correlation and, the equivocal status of C3 and C4 lesions. This study was undertaken to evaluate C3 and C4 categories and to correlate with a histopathological examination by core biopsy/excisional biopsy/lumpectomy specimen to determine their diagnostic reliability and direct the clinician in taking appropriate therapeutic interventions.

Materials and Methods

This perspective and retrospective study was undertaken for two years at the Department of Pathology of our institute. Patients with a clinical diagnosis of breast lump were included in the study. Aspiration was carried out using a 22G needle of 1-1.5 inch with a 20 ml disposable syringe under aseptic precautions. The aspiration was carried out by multiple punctures by oscillating movement of the needle in the same channel during each procedure to provide a high cell yield. After withdrawing the needle, the aspirated material was squeezed on the clean glass slides, and smears were prepared and then stained with May Grunwald Giemsa (MGG) after fixation.

All FNAs of breast masses were categorized according to NCI guidelines, and cases diagnosed as C3 and C4 were selected for further study. The category of C3 (atypical, probably benign) was assigned to those cases showing the overall characteristic of a benign aspirate with any or a combination of certain unusual features such as increased cellularity, nuclear pleomorphism, some loss in cellular cohesion, nuclear and cytoplasmic changes resulting from hormonal or treatment effects. An interpretation of C4 (suspicious for malignancy) was rendered to those cases showing highly atypical features, but still restraining us from forming a confident malignant diagnosis due to the presence of three main reasons-very scanty or poorly preserved specimen with some cells showing features of malignancy, absence of overtly malignant cells in an aspirate with a degree of abnormality more than those observed in category C3, and an aspirate displaying an overall benign pattern but with few cells showing distinct malignant features (7).

Histopathology findings were obtained through the processing of core biopsy, excisional biopsy, or lumpectomy specimens. FNA findings of smears labeled as C3 and C4 were compared with their respective histopathological diagnosis, and sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of category C4 were then calculated. Diagnostic values of C3 and C4 categories were determined by comparing the percentage of benign or malignant histological diagnosis in each category using the chi-square test of significance. The *P* of 0.05 or less was considered for statistical significance.

Results

A total of 602 FNA cytology of breast swellings were done during the study period of two years, out of which 60 (10%) had inadequate (C1) smears, and the remaining 542 smears were adequate (90%). Out of 542 satisfactory smears, 40 cases (7.4%) were assigned the category C3, and 32 cases (6%) were rendered the diagnosis of C4 comprising a sum of 72 cases (13.4%), respectively. Histopathological follow-up and correlation were available in 30 (75%) cases of C3 and 15 (47%) cases of category C4 and were selected for further study.

The patients under study were between 17-55 years of age. The maximum number of cases was in the age range between 21-30 years, *i.e.*, 33.3% (Table 1). There was a slight predominance of cases in the right-sided breast in our study. Out of 45 cases, the majority of them (n=27, 60%) were due to technical issues like low cellularity, smearing artifact, thick smear, poor fixation, and inadequate sampling, of which sampling error was the most attributable cause. Table 2 shows the distribution of various factors contributing to C3 and C4 lesions.

On histopathological follow-up, out of 30 cases of the C3 category, 22 (73.3%) cases turned out to be benign, and 8 (26.7%) cases revealed malignant diagnosis (Table 3, Figure 1) (Figure 2a and b). Among the benign histological diagnosis, 16 (72.7%) cases revealed the features of fibroadenoma, followed by 4

(18.2%) cases of proliferative breast disease and 2 (9.1%) cases of phyllodes tumor, while the most common malignant histologic subtype was found to be invasive ductal carcinoma (IDC) (n=6, 75%), followed by invasive lobular carcinoma (ILC) (n=2, 25%) (Table 4).

Among the 15 cases of the C4 category, 2 (13.3%) cases revealed benign findings (Figure 3a and b), and 13(86.7%) of the cases turned out to be malignant on histopathological correlation (Table 3, Figure 1). The benign histological diagnosis was fibroadenoma (n=1, 50%) and phyllodes tumor (n=1, 50%), while among the malignant histological diagnosis maximum cases were of IDC (n=12, 92.3%) followed by one (7.7%) case of papillary carcinoma (Table 4).

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the C4 category in the diagnosis of malignancy were 61.9%, 91.6%, 86.6%, and 73.3%, respectively. There was a considerable statistical difference between the number of benign and malignant outcomes for category C3 (73.3% benign) and C4 (86.7% malignant) (P<0.001)

Table 1. Distribution of Cases According to Age, in Reference to C3 and C4 Categories

Age group (years)	No. of cases (C3 + C4)	Percentage
11-20	04	8.9%
21-30	15	33.3%
31-40	12	26.7%
41-50	09	20.0%
51-60	05	11.1%
TOTAL	45	100%

Table 2. Distribution of Different Factors Contributing to C3 and C4 Category

Factors	No. of Cases (C3+C4)	Percentage
a. Technical Issues	27	60%
1. Paucicellular smears	09	33.3%
2. Smearing artifact or obscuration by drying artifact/haemorrhage	06	22.2%
3. Inadequate sampling	12	44.5%
b. Interpretative Issues	04	09%
c. Overlap of Cytologic Features of Benign & Malignant Conditions (True Gray Zone)	14	31%
Total	45	100%

Table 3. Cytohistological Correlation of C3 and C4 Lesions

Histological Diagnosis	Cytology Category C3	Cytology Category C4	Total
Benign (%)	22 (73.3%)	02 (13.3%)	24
Malignant (%)	08 (26.7%)	13 (86.7%)	21
Total (%)	30 (100.0%)	15 (100.0%)	45

Table 4. Cytomorphological Correlation of C3 (30) and C4 (15) Cases

Histological Diagnosis	C3	C4	TOTAL
Benign Cases	**22**	**02**	**24**
Fibroadenoma	16	01	17
Phyllodes Tumour	02	01	03
Proliferative breast disease	04	--	04
Malignant Cases	**08**	**13**	**21**
Invasive ductal carcinoma(NOS)	06	12	18
Invasive lobular carcinoma	02	--	02
Papillary carcinoma	--	01	01

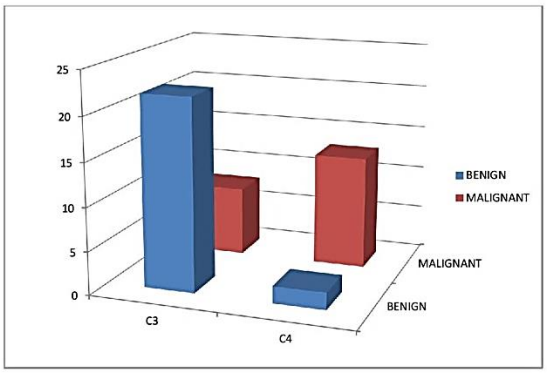

Figure 1. Bar diagram representing benign and malignant cases of C3 and C4 category

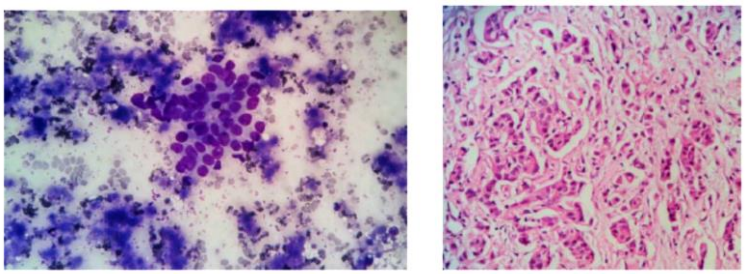

Figure 2. False-negative case – Atypical, probably benign (C3), turning out to be malignant. (a): FNAC category C3 showing clusters of ductal epithelial cells exhibiting mild discohesion and mild nuclear atypia along with few scattered myoepithelial cells. (MGG, 10 X 40), (b): Follow-up histopathological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

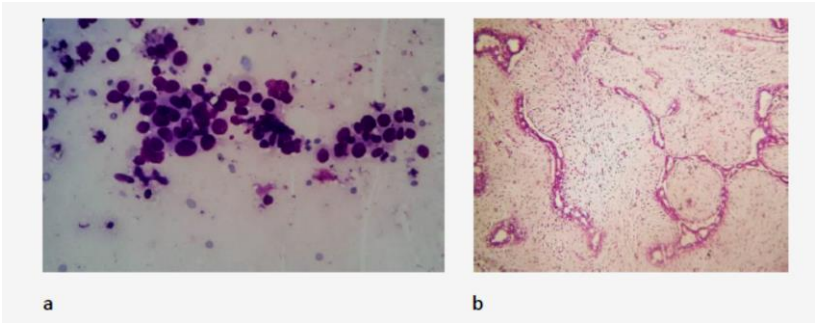

Figure 3. False-positive case – Suspicious, probably malignant (C4), turning out to be benign. (a): FNAC category C4 showing ductal epithelial cells exhibiting dyscohesion with moderate nuclear atypia. (MGG, 10 X 40), (b): Subsequent histopathological examination revealing features of typical fibroadenoma.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

Discussion

FNAC serves as a rapid, cost-effective, reliable tool for diagnosis of palpable breast lesions, and correlation with clinical examination and mammography provides an accurate diagnosis of breast cancer in around 99% of cases (8).

The patients in our study were between 17-55 years of age. The maximum number of cases was in the age range between 21-30 years, *i.e.*, 33.3%., almost similar to the study conducted by Madan M *et al.*, (9) and Yusuf I *et al.*, (10). There was a slight predominance of cases in the right breast in our study, comparable to the study done by Kumar R *et al.*, (11) and Rahman MZ *et al.*, (12) which showed predominant involvement of right-sided breast in their study. The majority of the cases

were due to technical issues *i.e*. (60%) in our observation of which inadequate sampling was the most attributable cause, comparable with Shabb NS *et al*., (7) and Kalhan S *et al*., (6), which showed technical limitations to be the major contributing factor in the diagnosis of C3 and C4 lesions.

Our study had 60 (10%) inadequate FNAC smears (C1), which fall within the range varying from 4.2% in a study by Nguansangiam S *et al*., (13) to 32% in a study conducted by Chaiwun B *et al*., (14), which might be either due to the lack of the technical experience or the nature of the lesion themselves.

Out of 542 satisfactory smears in our study, 40 cases (7.4%) were assigned the category C3, and 32 cases (6%) were rendered the diagnosis of C4 comprising a total of 72 cases (13.4%), respectively. This rate was higher than 6% reported by Chaiwun B *et al*., (15) and Kanhoush *et al.*, (5), 7.6% noted by Deb R A *et al*., (16), 8.4% registered by Goyal P *et al*., (17), and almost comparable to 13.3% documented by Arul P *et al*., (18), 12.2% reported by Khan A., (19) and 14.8% noted by Madan M *et al.*, (9). Our frequency of 13.4% lies within the range (6-14.8%) of the studies mentioned above, indicating that these equivocal categories C3 and C4 were not underused or overused in our study. The only exception was the study conducted by Yusuf I *et al*., (10), which reported a very high frequency of 23.5%.

On histopathological follow-up, among the C3 category, 8 (26.7%) cases revealed malignant diagnosis ("false negative") in our study, which is comparable with Goyal P *et al*., (37.5%) (17), Arul P *et al*., (35.7%) (18), and Madan M *et al*., (23%) (9) in which the result is in concordance with published reported values where the malignant diagnosis was rendered in 8.6-52% cases of C3 category with a major proportion reporting values >30% in their studies (5,16,20). The most common malignant histologic subtype was found to be invasive ductal carcinoma in our study, followed by invasive lobular carcinoma, comparable to recently reported studies (17,18). The small size of the tumor, low cellularity, and quantitative problems because of sampling errors during FNAC where we get only a few or no atypical cells in the smears examined are the main factors responsible for "false negative" results, of which the majority of them are due to inadequate sampling, observed in our study too. Less common factors include qualitative error where malignant cells are misinterpreted as benign, despite their presence in the smears examined, which encompasses malignancies of low nuclear grade, in situ carcinomas, and few special type tumors such as lobular carcinoma, scirrhous carcinoma, tubular carcinoma, mucinous carcinoma, and well-differentiated intra-cystic carcinoma (7,21,22). After histopathological correlation, the false-negative cases including IDC (n=6) and ILC (n=2) were reviewed rigorously, and all of these cases showed few dyscohesive clusters displaying mild to moderate nuclear pleomorphism and nuclear crowding against the background composed predominantly of monolayered cohesive epithelial sheets and clusters along with scattered bare nuclei. These accompanying benign features led to the underdiagnosing of these cases as C3 on FNAC. Correlation of FNAC outcomes with relevant clinical findings of the patient and mammography by the triple test can be beneficial in reducing the rate of false-negative cases and proper management of each patient (23).

Among the C4 category, 13 (86.7%) cases revealed the malignant diagnosis on histopathological correlation, which is in concordance with previously reported values ranging from 81% to 97% (5,14,16,20). Two (13.3%) cases turned out to be benign on histopathological follow-up, considered as "false positive," which includes 1 case each of fibroadenoma and phyllodes tumor in the present study. These cases displayed high cellularity, some dyscohesive clusters with moderate nuclear atypia, and cellular overlapping, which led to over-reporting of these cases as C4 on FNAC. Fibroadenoma has been documented as one of the most frequently misinterpreted lesions and hence "grey-zoned" according to several other studies (5,15,16,24). Some degree of nucleomegaly, nuclear atypia, and cellular dyscohesion combined with increased cellularity are often encountered in the smears of fibroadenoma, leading to diagnostic dilemmas, particularly in cases entitled "fibroadenoma with atypia" (24) as these cases have the probability of being misdiagnosed as low-grade carcinomas. Phyllodes tumor (PT) also represents an important grey zone category. The intracanalicular lining epithelium may exhibit significant atypia in a case of benign or malignant PT and can present as atypical detached cells in aspirates leading to an erroneous (false positive) interpretation of carcinoma on FNAC. However, stromal hypercellularity (including bipolar naked nuclei) and stromal atypia may be a more dependable feature than epithelial atypia, pointing to their histologic type (25).

C3 and C4 categorization of cases is imperative to recognize the groups of patients who are expected to have either benign (C3) or malignant (C4) consequences. Unnecessary surgical biopsies or any other intervention can be avoided in patients with C3

lesions if correlation with clinical examination and mammographic studies yields a benign result (*i.e.*, a negative triple test). A repeat FNAC or core needle biopsy (CNB) is advised after a minimum of 1 month, enabling the reparative changes to subside due to the previous aspiration. No surgical management is required if the second biopsy turns out to be benign (15,16). However, for patients with C4 lesions, histopathological examination and confirmation are highly recommended, due to greater chances of malignant outcomes in the C4 category, as proposed by some authors too (5,17,18).

There was a statistically significant difference in our study between the number of benign and malignant diagnoses for category C3 (73.3% benign) and C4 (86.7% malignant) (P<0.001), justifying the stratification of cases into equivocal categories C3 and C4 beneficial and thus mandatory.

FNAC is a simple, rapid, cost-effective, and accurate method to diagnose easily accessible breast swellings. However, one should be aware of its limitations as well. The equivocal categories C3 (atypical, probably benign) and C4(suspicious for malignancy) indicates the diagnostic problems experienced by the pathologist in classifying a few of the breast lesions accurately despite adequate sampling, while it directs a clinician to follow up with a repeat FNAC or CNB of those lesions after a reasonable period. Stratifying cases into C3 and C4 categories also minimizes the incidence of misdiagnosis in the straightforward diagnostic C2 (benign) and C5 (malignant) categories.

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