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Original Research Paper

Pathology

CORRELATION OF DIAGNOSTIC UTILITY OF FNAC AND CORE BIOPSY IN BREAST CARCINOMAS WITH IHC ANALYSIS OF SELECTED CASES

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ABSTRACT Breast cancer is a heterogeneous disease encompassing many morphological and molecular genetic entities like histological type, grade, hormone receptors and HER2 expression. They have been used to identify specific prognostic groups and predict response to the treatment. Fine Needle Aspiration Cytology (FNAC) is a relevant and important method to diagnose breast cancer, but technical problems such as limited cellularity, excessive air drying and artifactual mechanical disaggregation can potentially limit the interpretation, as well as contribute to a false-negative or a false-positive diagnosis of malignancy, respectively. The major current limitation of FNAC is the separation of atypical ductal hyperplasia (ADH) from ductal carcinoma in situ (DCIS) and differentiating DCIS from invasive carcinoma, which affect the patient's treatment. We carried out this study to compare between FNAC and semi-automated Core Needle Biopsy (CNB) to diagnose breast carcinoma. We found that on comparative analysis between FNAC and CNB in the diagnosis of breast carcinoma; sensitivity, negative predictive value, diagnostic accuracy were higher in case of CNB than those in case of FNAC. Immunohistochemistry (IHC) was done on tissues from CNB of all the diagnosed malignant cases and were categorised through ER,PR,HER2 and Ki67 staining in four group as Luminal A, Luminal B, HER2 Rich and Basal Like taking Ki67 cut up mark at 14%.We found that HER2 Positive primary tumour apparently was more associated with higher Grade and increased nodal involvement.

KEYWORDS : Fine needle aspiration (FNAC), Core needle biopsy (CNB), Immunohistochemistry (IHC), Breast carcinoma.

INTRODUCTION:

Palpable breast mass is a common problem in female patients. The diagnostic delays of breast cancer occur due to generally low index of suspicion. With rising incidence and awareness, breast cancer is the commonest cancer in urban Indian females, and the second most common in the rural Indian women. [1]

More than 80 percent of breast cancer is thought to be associated with environmental factors that include exposure to contaminants, lifestyle and diet, and exposure to ionizing radiation. [2] Indian breast cancer patients present with advanced disease stage and have numerous poor prognostic factors such as large tumor, lymph node metastases, high pathological grade and poor hormone receptors status. Besides, there is poor access to high-quality multi-modality treatment facilities for many patients. [3]

Breast cancer is a heterogeneous disease encompassing many morphological and molecular genetic entities. Several markers like histological type, histological grade, hormone receptors and HER2 expression have been used to identify and specific prognostic groups and predict response to the treatment. FNAC is a relevant and important method to diagnose breast cancer, but technical problems such as limited cellularity, excessive air drying and/or artifactual mechanical disaggregation can potentially limit the interpretation, as well as contribute to a false-negative or a false-positive diagnosis of malignancy, respectively. These limitations have contributed to increase in the use of CNB. Advantages of CNB is that it provides adequate tissue for definitive histological diagnosis, distinguish between invasive cancer and carcinoma in situ patients for whom FNAC is inconclusive; and breast lesions with micro calcifications; and for research purpose tissue banking specimen CNB is of great benefit [4]. In a case of palpable breast lump radiological imaging in combination with needle biopsy reduces the need for unnecessary surgical excision of benign breast lesion.

Some studies suggest that the gold standard for the evaluation of these biomarkers must include IHC in both the CNB and the Surgically Operated specimens(SOS) and most authors emphasize the importance of retesting hormone negative CNB biopsies.[5–8] HER-2 over expression is also associated with a more aggressive disease and poorer prognosis, playing an important role in treatment decisions regarding the use of a trastuzumab regimen, [9] a target based therapy that employs a monoclonal antibody that interferes with the HER-2/neu receptor. [10].

MATERIAL AND METHODS:

The study was conducted in the Department of Pathology from 1st January, 2017 to 30th June, 2018. FNAC was done in all female patients followed by CNB in the same patients who attended the Surgery OPD with suspected breast malignancy. Then we compared the result of FNAC and CNB in the light of histopathological examination of post-operative Specimens which is regarded as gold standard. IHC was also done with special reference to high grade cases. Patient particulars like Age, Sex, Occupation, Weight; personal history like Age at menarche, Marital status, Age at marriage, Parity, Menopausal status, History of OCP use, Addiction, Significant family history in first degree relatives were noted and followed by clinical examination including Tumor size, Mobility, Palpable lymph nodes. Findings of radiological investig ations like Ultrasonography and Mammography were noted. Histopathological findings were recorded like Tumour characteristics (size, morphology, nipple/skin invasion and grade including presence of necrosis and lympho-vascular invasion), Stage, Nodal status, location and margin status. In FNAC cases were categorised as BENIGN, SUSPICIOUS and MALIGNANT. In CNB cases were categorised as BENIGN, ATYPICAL, SUSPICIOUS AND MALIGNANT. Histopat holo gical Diagnosis (HPD) under microscope of all the study cases were categorised as above and HPD was taken as GOLD STANDARD for evaluation of all the cases. Routine staining of all the sections were done by Hematoxylin and Eosin (H&E) stain. HPD was obtained from tissue sections from surgically

excised specimens (SES) were categorised into two broad groups: malignancy- (carcinoma) diagnosed; and Malignancy(carcinoma)- not diagnosed (including all benign, with atypia, suspicious) following conventional histological protocol for individual diagnosis. IHC analysis was done with special reference to high grade cases : ER, PR and Her2/neu, Ki67 of those patients. Previously operated and recurrent cases were excluded. Those cases who received chemotherapy after CNB and before surgery were also excluded from the study.

Immunohistochemistry procedure:

1. The Poly-L-Lysine coated representative sections were labeled
2. Sections were placed within Hot Xylene for 20 mins and then successively passed through following solutions.

a. Xylene I 10 min
b. Xylene II 10 min
c. 100% Alcohol 5 min
d. 90% Alcohol 5 min
e. 70% Alcohol 5 min

3. Sections were washed in tap water 1-2 min each.
4. The slides were then arranged in a slide cradle. Four sections of each case were taken. A domestic pressure cooker of 2 liters size was filled with one liter of TRIS/EDTA buffer (pH 9.0) and the slide cradle was dipped in this solution. The pressure cooker was removed from heat after 1st whistle and kept under tap water for 30 to 45 min i.e., till it reached room temperature. One slide for one antibody of each case was selected.
5. The sections were removed from TRIS/EDTA buffer and washed in TRIS (wash) buffer 3 changes for 1min each.
6. Slides were removed from TRIS buffer and extra buffer tapped off. Endogenous peroxide quenching was performed by adding 3% H2O2 in distilled water. The sections were incubated with 3% H2O2 for 15 mins. Later sections were washed in TRIS buffer 3 changes, 2 min each.
7. Excess of TRIS buffer was tapped off and incubated with respective primary antibodies of known dilution and incubated for 45 min -1hr in humid chamber.
8. The slides were washed in TRIS buffer, 3 changes of 1min each. Secondary antibody was then added to the sections and incubated for 30 min in a humid chamber. The slides were washed in TRIS buffer 3 changes, 1 min each.
9. DAB solution was prepared freshly and added to sections for 10 mins and monitored for the development of colour.
10. The sections were washed in distilled water and counter-stainined with Harris's haematoxylin for 45 sec. Blueing was done by washing the sections in running tap water.
11. Sections were dehydrated using ascending grades of alcohol, cleared in xylene and mounted with DPX.

Each batch contained a positive control i.e., where staining state is known and a negative control i.e, to pick up the background staining. The sections of negative control were incubated with TRIS buffer instead of primary antibody.

IHC evaluation- The staining was evaluated on the invasive component only. Best-preserved and best-stained areas of the sections were assessed. . Nuclear staining was assessed for ER and PR; while membrane staining was assessed for Her 2. A score for the proportion of stained cells (0 = no nuclear staining, 1 = <1% nuclear staining, 2 = 1-10% nuclear staining, 3 = 11-33% nuclear staining, 4 = 34-66% nuclear staining and 5 = 67-100% nuclear staining) and the intensity of staining (0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = strong staining) were assigned to each tumour. **The proportion score (PS) and intensity score (IS)** are added together for a total score, which ranged from 0 to 8.

Total score: PS + IS Interpretation

0 to 2- Negative; 3 to 8- Positive

HER-2 status was considered positive whenever IHC score is 3+ and negative for

score 1+. Score 2+ was considered as equivocal and excluded in this study.

Only nuclear staining (plus mitotic figures which are stained by Ki67) were incorporated into the Ki67 score that is defined as the percentage of positively stained cells on total of 1000 tumor cells counted. Positive Ki-67 value was assigned when we observed ≥ 14% in the IHC preparation, whereas a negative value was determined when this was <14%.

RESULTS AND ANALYSIS:

A prospective observational study with a cross-sectional study design was conducted with 70 cases of suspected breast malignancy. 61% were in their premenopausal and 39% were in their postmenopausal status. Due to early age of marriage in India, almost 93% of the study population was parous in our study. The majority of the patients of the study population i.e., 59% of all 70 patients were non-overweight**.** In the present study, maximum numbers of cases were in the age range of 31-40 years (28/70, 40% cases) with a mean age of 45.18 years.

In this study majority of the patients i.e., 59% were not OCP user. There is family history of breast cancer in five patients (7%). In this study 'HIGH STAGE DISEASE' i.e., STAGE II was 40%, STAGE III was 50.77% and STAGE IV was 3.08%.

Two cases were found to have skeletal metastases as per bone scan. First case was Grade II,size-9.6cm, - pT3N2M1with ER+,PR+,HER2-,Ki67-13% and second case was Infiltrating Carcinoma (No Special Type) of Gr III,size-10.6cm, -pT3N2M1 with ER-,PR-,HER2-,Ki67-70% (Triple Negative, Basal Like). Both cases got Neo Adjuvant Chemotherapy (NACT) and in those cases IHC was done from CNB.

With increasing Tumour size (> 5cm) the Number of involved Node increased; with T1 tumour size (≤ 2cm) N1 was 12.31% and N2 was 0% but with T3 (>5cm) N1 became 30.77% and N2 became 23.08%. Nodal involvement also increased with increasing grade of the tumour.

Within 65 cases ER+ was 86.15% (56 cases) and ER- was 13.85% (09 cases); PR+ was 72.31% (47 cases) and PR- was 27.69% (18 cases); HER2+ was 18.46% (12 cases) and HER2- was 81.54% (53 cases); Ki67 was < 14% in 64.62% (42 cases) and >14% in 35.38% (22 cases).

Using IHC analysis as surrogate marker for the molecular subtypes of breast cancer, the 65 confirmed malignant cases under study were subtyped as below.

LUMINAL A- 42 cases (64.61%)
LUMINAL B- 14 cases (21.54%)
HER2 ENRICHED- 04 cases (5.9%)
BASAL LIKE- 05 cases (7.35%)

Table 1. Distribution of all the patients as per their final HPE diagnostic category [further allocated into two broad groups as MALIGNANCY–NOT DIAGNOSED and MALIGNANCY– DIAGNOSED] of surgically operated specimens in accordance with different cytologic FNAC category (n = 70).

Table 1a		Diagnostic HPE		
FNAC		Malignancy Diagnosed	Malignancy Non-Diagnosed	Total
	Malignancy Diagnosed	50(TP)	0(FP)	50

	Malignancy Non- Diagnosed (Suspicious +Benign)	15(FN)	5(TN)	20
Table 1b		Diagnostic HPE		
CNB		Malignancy Diagnosed	Malignancy Non-Diagnosed	Total
	Malignancy Diagnosed	60(TP)	0(FP)	60
	Malignancy Non-Diagnosed (Benign+Atypia+suspicious)	5(FN)	5(TN)	10
	Total	65	5	70

FNAC showed sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy were 76.92%, 100%, 100%, 25% and 78.57% respectively in diagnosing carcinoma.

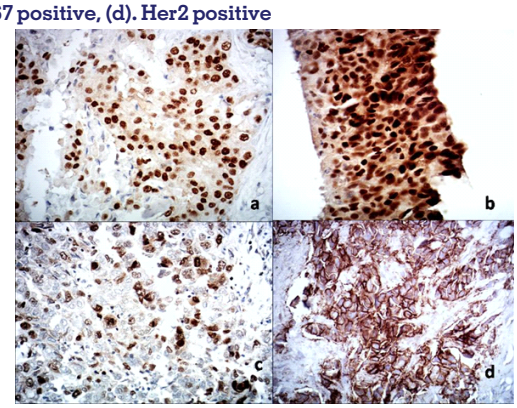
CNB had sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of 92.30%, 100%, 100%, 50% and 92.86% respectively. All patients who were categorized as malignant by CNB were confirmed as malignant by subsequent HPE of surgically operated specimens (100% positive predictive value).

So, on comparative analysis between FNAC and CNB in the diagnosis of breast carcinoma; sensitivity, negative predictive value, diagnostic accuracy were higher in case of CNB than those in case of FNAC. Detection of false negative cases was also lower in CNB assessment. Regarding specificity, positive predictive value, and detection of true negative cases (i.e. which patients really did not have definite malignant lesion); both the procedures turned out to be similar.

Table 2. Correlation of Hormone Receptor status with Tumor Grade.

	ER		PR		HER2	
	Positive	Negative	Positive	Negative	Positive	Negative
GR I	29 (44.61%)	0	28 (43.08%)	1 (1.54%)	3 (4.61%)	26 (40%)
GR II	21 (32.31%)	1 (1.54%)	16 (24.61%)	6 (9.23%)	3 (4.61%)	19 (29.23%)
GR III	6 (9.23%)	8 (12.31%)	3 (4.61)	11 (16.92%)	6 (9.23%)	8 (12.31%)

Fig 1: IHC stain x 400, (a). ER positive, (b) PR positive, (c). Ki67 positive, (d). Her2 positive



DISCUSSION:

Several studies have been conducted to compare the role of FNAC and CNB [11,12]. In our study, carcinoma was most commonly diagnosed in the age group of 31-40 years (40%) followed by 41-50 years (35.72%) and most are premenopausal (61%). Khemka et al. expressed that the peak incidence of breast carcinoma was between 40-44 years [11]. As per statistics from Breast Cancer India (BCI), the average age of developing a breast cancer has undergone a significant shift over last few decades. An increasing numbers of patients are in the 25 to 40 years of age [13]. 52.85% presented with breast lumps in upper outer quadrant of breast followed by central quadrant (18.51%). Hussain in his series had 58% of patients in whom upper outer quadrant was involved in breast lumps [14]. Early breast cancers situated in central/internal quadrants have a worse prognosis compared with those in lateral quadrants [15]. In our study, among 65 patients, size of the lumps ranged from 2cm to 12 cm. Of all 65 patients having malignancy 63.08% had their breast lesions >5cm of size. Numerous studies have shown that survival decreases with increasing tumour size and that there is a coincidental rise in the frequency of axillary nodal metastases [16]. In our study, 7.7% show nipple retraction, peau d'orange and ulcerative skin change. Axillary lymph nodes are usually the first anatomic site to be involved by metastases in patients with breast carcinoma. The regional lymph node status is the most important prognostic factor in patients with breast cancer and adjuvant therapy protocol after excision of the primary tumour is determined according to lymph node status [17]. Studies by Homesh NA et al, Usami S et al, to compare CNB & FNAC have reported very high sensitivity (91–99%), specificity (96–100%), positive predictive value (100%), and negative predictive value (100%) for CNB which are better than results for FNAC for both palpable and nonpalpable lesions [18,19]. In our study; sensitivity, negative predictive value, diagnostic accuracy were higher in case of CNB than those in case of FNAC. Detection of false negative cases was also lower in CNB assessment. Regarding specificity, positive predictive value, and detection of true negative cases (i.e. which patients really did not have definite malignant lesion); both the procedures turned out to be similar.

Though data from developed countries suggested that most of the breast cancer patients do not have any lymph node metastasis, Indian studies have documented higher percentages of lymph nodal involvement in breast cancer patients. [20-22] LVI is associated with poorer prognosis. [23,24] In the present study 20% cases showed lymphov ascular invasion.

One study revealed that tumor necrosis is predictor of early recurrence. [25] In the present study 29.23% cases showed tumor necrosis.

Tumor grade is one of the prognostic factors in the breast cancer. Tumors expressing higher grade tend to carry poor prognosis. In this study, grade II tumor constituted the highest number of cases (49%) followed by grade III and grade I. Similar findings were recorded in other studies in India.[26] Grading has also been done on CNB specimen and it showed comparable results with operated specimen's grading.

Histology as a prognostic factor has been well documented. IDC-NST is the most prevalent histological type, accounting for 80- 85% of all malignant breast neoplasms. [27] In agreement with previous studies, [28] in the present study- the most common histological type was Invasive carcinoma-NST (83.08%), followed by Invasive lobular carcinoma (4.61%). Two cases were Medullary carcinoma, Mucinous carcinoma and Neuroendocrine carcinoma (3.08% each) and Others cases were metaplastic carcinoma and malignant phyllodes. An accurate preoperative diagnosis of breast lesions with IHC is crucial for optimal individualized treatment decisions. The presence or absence of biomarkers determines the necessity of endocrine manipulation therapy or a trastuzumab regimen. Thus early diagnosis and treatment reduce the cancer death. Using immunohistochemistry as surrogate marker for the molecular subtypes of breast cancer the 65 confirmed malignant cases under study were subtyped as below:

LUMINAL A- 42 cases (64.61%); LUMINAL B- 14 cases

(21.54%); HER2 ENRICHED- 04 cases (5.9%); BASAL LIKE- 05 cases (7.35%). This study is nearer to the study of A Spitale, et al [29].

CONCLUSION:

FNAC is a rapid, less complicated, economical, reliable and relevant method for the preoperative pathological diagnosis of breast carcinoma in a developing nation like ours. With high sensitivity and specificity, most malignant breast lesions can be reliably diagnosed by FNAC. If the initial FNAC is inadequate, CNB can be a useful second line method of pathological diagnosis in order to minimize the chance of missed diagnosis of breast cancer. One should be mindful of the limitations of each technique and the choice between FNAC and CNB should be individualized for the patient. IHC can be done in a reliable and accurate manner from CNB samples for those patients undergoing NACT. Surgically operated biopsy should be the last option to obtain a pathological diagnosis.

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