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UTILITY OF IMMUNOCYTOCHEMISTRY AS AN ADJUNCT TOOL TO FNAC IN ROUTINE CYTOLOGICAL PRACTICES.

Pathology

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ABSTRACT

Background: Immunocytochemistry (ICC) can be used as adjunct tool in routine cytological practices applied on cell blocks from FNAC material. **Results:** This is a retrospective one year study involving 46 out of 965 patients who underwent FNAC with ICC as an adjunct tool. We had cytological-histopathological correlation in 20 out of 22 cases (90.9%). The most common cytological diagnosis was metastatic carcinomas (52.2%). Biomarker studies for breast cancer were done in 10 cases with biopsy correlation in 5 cases showing concordance rate of 100%, 60% and 80% in ER, PR and HER 2 neu respectively. **Conclusion:** ICC is a sensitive and reliable adjunct diagnostic tool to FNAC in routine cytological practices while evaluating for neoplastic lesions and detecting primary site lesions in unknown primaries.

KEYWORDS

Immunocytochemistry, FNAC, Cell block

Introduction:

Fine needle aspiration cytology (FNAC) is a routine diagnostic tool performed in the outpatient procedure room of many institutions. Few of the cases like undifferentiated neoplasms can be challenging to give definite diagnosis by morphological findings alone which may require supporting immunocytochemistry (ICC) as an adjunct diagnostic tool. [1] FNAC material can be used to prepare cell blocks which is a mini formalin fixed paraffin embedded (FFPE) biopsy, helping us to prepare the cytological material for routine ICC and molecular studies to reach to a accurate diagnosis.[2]

FNAC can also play a key role in the administration of neoadjuvant chemotherapy for management of advanced breast cancers by assessing the biomarkers like estrogen receptor (ER), progesterone receptor (PR) and Her 2 neu receptor (HER 2) using ICC as an ancillary technique to routine cytological practices. Hormone studies using ICC can further help us to understand if the biomarker profile is the same or altered which has an impact on overall patient management.[3,4]

Evaluation of regional lymph nodes and suspicious metastatic nodules at distant sites can be done using FNAC with ICC complementing the morphological findings reaching a definite diagnosis for faster and effective patient management especially among cancer patients.[5]

The aim of our study was to study the utility of immunocytochemistry as an adjunct tool to FNAC in routine cytological practices. The study objectives are

1. To study the spectrum of cytological lesions in FNAC using ICC as an adjunct diagnostic tool.
2. To compare the diagnostic accuracy of cytological aspirates using ICC in FNAC in comparison to the gold standard histopathological diagnosis from biopsy specimens.

Methodology:

This s a one year retrospective study carried out in the department of oncopathology of a cancer centre from 1st January, 2016 to 31st December, 2016. The study sample included all patients who underwent FNAC with ICC as an adjunct tool. All the demographic data and clinical details were obtained from patient's record file retrieved from the medical record department of the cancer institute.

The cytological evaluation was carried out by screening of the routine slides (May Grunwald Geimsa and Papanicolaou stained slides) inclusive of the representative ICC slides. The histopathological evaluation was performed by reviewing the representative histopathological slides of the selected cases which were compared respectively.

The inclusion criteria included all patients who underwent FNAC with ICC as an adjunct diagnostic tool to aid in the cytological diagnosis over the last one year.

The exclusion criteria included patients who underwent FNAC without supporting ICC and FNAC with inconclusive reports due to poor yield. Statistical studies were carried out using descriptive statistical tools to test the discrepancy between the cytological diagnosis from FNAC and histological diagnosis from biopsy.

Results:

There were 46 out of 965 patients who underwent FNAC with ICC as an adjunct tool in the outpatient procedure room of rural cancer centre over one year. Patient's age varied from 16 years to 88 years with a male: female sex ratio of 1:1. The cytological diagnosis obtained after immunocytochemistry studies was confirmed by histopathological evaluation and immunohistochemistry when required. Few of the antibodies frequently used to perform immunocytochemistry are Pancytokeratin (CK), Cytokeratin 7 (CK 7), Thyroid Transcription Factor 1 (TTF-1), p63, Cytokeratin 20 (CK 20), ER, PR, Leucocyte Common antigen (LCA/CD 45), HER 2 neu, CD 3 and CD 20.

We had histopathological biopsies for correlation in 22 cases. Our cytological diagnosis correlated in 20 out of 22 cases (90.9%) cases. The breakup of cases is highlighted in Table 1. The rest of the patients did not undergo biopsy for few reasons like advanced malignancy (stage 4) and old age, advanced disease (stage 4) with poor ECOG Scale of Performance status (PS) score 3 and 4, palliative RT, reluctance to undergo further treatment, not able to tolerate to chemotherapy and radiotherapy, hence treatment discontinued and referred to nearest palliative clinic with no further intervention.

The most common sites from which FNAC was done were lymph nodes (55%). Few of the other sites were breast (15%), thyroid (9%), bone (7%), soft tissue and intra oral (5% each), pelvic mass and skin (2% each).

Among the 46 patients, the most common cytological diagnosis were metastatic carcinomas (52.2%) followed by definite primary carcinomas from breast, thyroid and skin (23.9%); lymphomas (13.0%) and sarcomas (4.4%) (Fig 1).

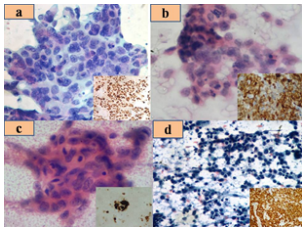

Fig 1: (a) Metastatic lung adenocarcinoma (PAP, x400), inset: TTF-1

nuclear positivity (IHC, x400); b) Metastatic malignant melanoma (PAP, x400), inset: HMB-45 positivity (IHC, x400);, (c) Metastatic squamous cell carcinoma (PAP, x400), inset: p63 nuclear positivity (IHC, x400);, (d) Non Hodgkin lymphoma (PAP, x400), inset: Diffuse CD 20 positivity (IHC, x400)

Table 1: Distribution of patients with cytological diagnosis including ICC studies and corresponding histopathological findings

Age/ sex	Site	Cytological diagnosis (LM)	ICC	Cytological diagnosis (LM+ICC)	Histopathological diagnosis (gold standard)
79/M	Inguinal LN	Metastatic PD Carcinoma	CK+, p63+	Metastatic PD SCC	PD SCC
70/M	Cervical LN	Metastatic PD Carcinoma	CK+,CK 7+, CK20-	Metastatic PD Carcinoma possibly lung	Metastatic SCC
75/F	Inguinal LN	Metastatic PD neoplasm	HMB 45+, S100+, CK-, LCA-	Metastatic malignant melanoma	Malignant melanoma, amelanotic variant
70/F	Cervical LN	Metastatic PD carcinoma	CK+, p63+	Metastatic PD SCC	Metastatic MD SCC
71/M	Vallecula	Non Hodgkin lymphoma	LCA+ (diffuse), CD 20+ (diffuse), CD 3-	B-NHL	Low grade B-NHL
67/M	Cervical LN	Metastatic adenocarcinoma	CK+,CK7+,CK 20+	Metastatic adenocarcinoma of Urinary bladder origin	Metastatic PD carcinoma of bladder origin
65/M	Cervical LN	Metastatic PD neoplasm	CK-, LCA-	Metastatic PD neoplasm	Metastatic large cell NEC
56/F	Breast	Carcinoma breast	ER+, PR-, HER 2-	Carcinoma breast ER+, PR-, HER 2-	IDC ER+, PR-, HER 2 (Eq)
50/F	Cervical LN	Metastatic PD carcinoma	CK+, p63+	Metastatic PD SCC	Dysplastic Squamous cells, Palliative treatment
55/M	Cervical LN	Metastatic PD carcinoma	CK+, p63+, LCA-	Metastatic PD SCC	MD SCC
70/F	Breast	Carcinoma breast	ER+, PR-, HER 2 (+)	Carcinoma breast ER+, PR-, HER 2(+)	IDC ER+, PR+, HER 2 (+)
59/M	Cervical LN	Non Hodgkin lymphoma	CD20+, CD3-,	B-NHL	B-NHL, high grade
62/M	Cervical LN	Metastatic PD neoplasm	CK+, LCA-	Metastatic PD carcinoma	Metastatic RCC
56/F	Breast	Carcinoma breast	ER+, PR-, HER 2(Eq)	Carcinoma breast ER+, PR-, HER 2 (Eq)	IDC ER+, PR-, HER 2 (+)
67/M	Scalp	Recurrant PD carcinoma	p63+	Recurrant PD SCC	Cutaneous SCC
49/F	Cervical LN	Metastatic carcinoma breast	ER-, PR-, HER 2(+)	Metastatic Carcinoma breast ER-, PR-, HER 2 (+)	Metastatic carcinoma breast ER-, PR+, HER 2 (+)
57/F	Medial canthus	NHL relapse	CD20+, CD3-	B-NHL, relapse	B-NHL, relapse
55/F	Breast	Metastatic breast carcinoma	ER+, PR-, HER2 (+)	Metastatic breast carcinoma ER+, PR-, HER2 (+)	k/c/o Carcinoma breast, 15 yrs back (No record of biomarker profile)
28/M	Thyroid, cervical LN	Carcinoma thyroid with metastasis possibly medullary origin	TTF-1+, CEA+	Medullary thyroid carcinoma with nodal metastasis	Medullary thyroid carcinoma with nodal metastasis
53/M	Breast	Plasmacytoma, to rule out carcinoma	CK+, ER+, PR+, HER2(+), E- cadherin+ CD138 (focally +)	Carcinoma breast ER+, PR+, HER2(+)	IDC ER+, PR+, HER2+
50/F	Cervical LN	Metastatic PD carcinoma	CK+, p16+, LCA-, TTF-1(-)	Metastatic PD carcinoma of cervical origin	PD non kerainizing SCC, cervix, p63+, p16+
68/M	Rib, Thigh	Metastatic adenocarcinoma	TTF-1 (-)	Metastatic adenocarcinoma	WD lung adenocarcinoma Ck7+, TTF-1 (focal weak +)

LM, light microscopy; LN, lymph node; PD, poorly differentiated; MD, moderately differentiated; WD, well differentiated; RCC, renal cell carcinoma; Eq, Equivocal; SCC, Squamous cell carcinoma; NHL, Non Hodgkin lymphoma; IDC, Infiltrating ductal carcinoma

The primary site of malignant neoplasm was obtained using immuocytochemistry as adjunct tool to FNAC in 19 cases (41.3%). Among the various primary site of origin of tumour, lung is the most common primary site (27%) followed by thyroid (16%), breast (11%) and cervix (6%).

Among the lymph node aspirates, there was histopathological correlation done in 15 out of 30 cases with a concordance of 100%. Biomarker studies for breast cancer were done in 10 cases. ASCO/CAP 2013 guidelines for HER 2 neu scoring and Allred score for ER and PR interpretation were used.[6,7] There was biopsy correlation in 5 cases showing concordance rate of 100%, 60% and 80% in ER, PR and HER 2 neu respectively.

There was a male patient, known case of multiple myeloma, presenting with right breast lump. FNAC revealed sheets of plasmacytoid cells, occasional binucleate forms raising the possibility of plasmacytoma. ICC on cell block preparation revealed diffuse positivity of CK, E-cadherin and scattered occasional positivity of CD 138. Biomarker studies for carcinoma breast were done showing triple positivity (ER, PR and HER 2neu positivity) (Fig 2). The cytological findings correlated with the histopathological findings from the breast biopsy with similar immunohistochemistry findings.

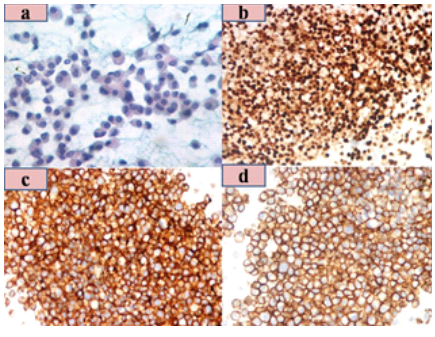

Fig 2: (a) Male breast carcinoma (IDC) with plasmacytoid differentiation (PAP, x400), b) ER nuclear positivity, Allred score: 8 (IHC, x400), (c) HER 2 neu strong, complete membrane positivity (score 3+) (IHC, x400), (d) E- cadherin positivity (IHC, x400)

Discussion: Fine needle aspiration cytology has gained popularity in recent years, especially with ICC as an adjunct tool, being a simple, minimally invasive outpatient procedure carried out across many hospitals in India from small nursing homes to large tertiary care

hospitals and cancer centre. Sometimes difficult aspirates may pose diagnostic challenges for the pathologist in giving a definite diagnosis, critical in the management of a disease condition, even more in cases of suspicion of malignancy.

There are variety of ancillary techniques like immunocytochemistry, molecular studies and electron microscopy which can be carried out on the cell block material obtaining satisfactory results providing more definite diagnosis, thereby initiating definite treatment without much delay.

Undifferentiated neoplasm can pose a serious diagnostic dilemma for the pathologist. ICC can uplift the burden upon the pathologist by opening the avenue for a variety of antibodies to help differentiate from various tumours entities.[1]

FNAC may at times be the only investigative tool carried out in the diagnosis and management of metastatic malignancy. ICC studies are at times used as an ancillary tool to identify the possible primary site and make a precise diagnosis especially for metastatic work up of diseases.[8] Cell block preparation were suitable for ICC due to limited background staining, comparable morphological findings with surgical biopsy specimens and reproducible, accurate and reliable immunostaining patterns.[9]

In our study, there were 46 out of 965 patients who underwent FNAC with ICC as an ancillary tool in the outpatient procedure room. Patient's age varied from 16 years to 88 years with a male: female sex ratio of 1:1. Mandal et.al and Mutreja et.al had a slightly larger study population (64 & 78 cases respectively) with a male preponderance 1.2:1.[1,9]

In the current study, there were 20 out of 22 cases (90.9%) with histopathological correlation which was slightly higher as compared to Mandal et.al who had 88.9% cases with histopathological correlation.[1] In the present study, the most common primary sites of malignant neoplasm based on the ICC findings were lung (26.7%) followed by thyroid (15.8%). Mandal et.al documented head and neck lesions (30.5%) to be the most common site followed by soft tissue (28%) and lung (22%).[1] Interestingly, in our study, the diagnostic accuracy combining both light microscopy and ICC as adjunct tool was 90.9% which was higher as compared to the study by Mutreja et.al who recorded 67.6% diagnostic accuracy.[9]

Based on the ICC results, metastatic carcinomas accounted for half the cases (52.2%) which was slightly higher as comparing with Mutreja et.al findings which showed 39% (16/41 cases).[9]

In our study, we had 10 patients diagnosed with invasive breast cancer on FNAC and biomarker studies (ER, PR and HER 2 neu) were performed on the representative cell blocks. We had a concordance rate of 100%, 60% and 80% in ER, PR and HER 2 neu respectively. Geethamala et.al did a comparative study between ICC and IHC on breast carcinoma and found a concordance of 98%, 97% and 89% in ER, PR and HER 2 neu respectively. Though the number of cases in our study is very less, the ER and HER 2 neu results are comparable with Geethamala et.al findings.[4] Radhika et.al found much lower concordance rate of 50% and 29% for ER and PR respectively while comparing ICC with IHC results in breast carcinoma.[3]

Determining the appropriate fixative and processing techniques plays a crucial role in preservation of the antigens thereby better detection rate of biomarkers and accurate interpretation. In our study, we used 10% buffered formalin as fixative and kept the tissue in formalin overnight at 40C. Antigen retrieval can be performed using pressure cooker or microwave, though microwave is found to give optimal results.[10]

Mitteldorf et.al did not find ICC to be complementary to routine FNAC.[11] False positive results can be encountered in routine practice while handling cell blocks for ICC studies especially in the setting of poor fixation, drying artifact, necrotic background, cross reaction by antibodies, inadequate peroxidase blocking and lower levels of antibody specificity. Conversely, false negative results are encountered due to inadequate antigen retrieval, prolonged fixation, denaturation and poor antigen preservation.(1,9) ICC is used as an ancillary tool with more usefulness in aspirate samples.[12]

In the current study, we concluded that metastatic carcinoma was the most common cytological diagnosis. Lung was the most common primary site of origin of tumour using ICC as an adjunct tool. Biomarker studies were carried out on the breast carcinoma cases using cell block preparation with ICC giving promising and satisfactory results. ICC is a sensitive and reliable adjunct diagnostic tool to FNAC in routine cytological practices while evaluating for neoplastic lesions and detecting primary site lesions in unknown primaries.

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