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Immunohistochemical expression of AE1, AE3 on cell blocks of metastatic lymphnodes

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Abstract: Overview: Cancer poses a significant global threat[1]. Lymph nodal metastasis is a crucial prognostic factor, and its detection presents a challenge for pathologists.

Aims: The aims of this study are as follows: to determine the common sites affected by metastatic lymphadenopathy, to evaluate the effectiveness of the cell block technique in preserving cell morphology compared to conventional fine needle aspiration (FNA) samples, to assess the preservation of immunohistochemical properties using the cell block technique, and to determine the role of immunohistochemistry in cell block preparations of malignant breast neoplasms for making a final diagnosis.

Materials and Methods: Immunohistochemistry using AE1 and AE3 markers was performed on sections made from cell blocks obtained through the fine needle aspiration technique. Cell block preparation involved a mixture of 9 parts ethyl alcohol and 1 part 10% formalin, followed by hematoxylin and eosin staining. Two pathologists independently analyzed H&E-stained smears and immunocytochemistry results, and the findings were calculated.

Results: In the present study, pan CK positivity was observed in 31 out of 48 cases, of which 25 were squamous cell carcinomas, 2 were suspicious for malignancy, and 2 were other cases.

Conclusion: The use of Pan CK (AE1/AE3) markers demonstrated a significant percentage of positivity in identifying squamous differentiation. This panel of markers, along with others, can be employed for epithelial differentiation and can assist in neoadjuvant therapy and patient care.

Keywords: Cancer; Lymph Nodal Metastasis; Cell Block Technique; Immunohistochemistry; Squamous Differentiation.

1. Introduction

Cancer is the most threatening problem worldwide [1]. Lymph nodal metastasis is the most important prognostic factor. Detection of metastasis is challenging for pathologists and surgical oncologists. Fine needle aspiration cytology (FNAC) is a procedure to get material from the tissues.

Cell block is a mini formalin-fixed, paraffin-embedded biopsy obtained from FNA or fluid sediment. It avoids the biopsy & preserves the nuclear-cytoplasmic architectural details. It provides almost 10 to 12 sections [2–4]. They can be useful for ancillary studies like IHC, electron microscopy, molecular studies which add diagnostic accuracy. Nowadays, immunohistochemistry of (AE1&AE3) plays a significant role in the identification of metastasis in lymph nodes.

AE1&AE3 are a mixture of 2 different clones of anticytokeratin monoclonal antibodies. They are broad-spectrum cytokeratin markers for cytokeratin 1-8, 10, 14-16, and 19. On immunohistochemistry, AE1&AE3 show both cytoplasmic & membranous positivity. AE1&AE3 are used to confirm or rule out the epithelial nature of tissue, tumors, & their components. They can be used in discrepancies like displaced epithelium by recent biopsy instead of true metastasis, hyalinized cytokeratin particles without tumor cell nuclei mimic isolated tumor cells, identification of tumor cells in significant cautery artifacts or dense inflammatory infiltrate obscuring infiltrating cancer cells [5,6].

1.1. Aims

To determine the age and sex distribution of patients presenting with metastatic lymphadenopathy. To determine the common sites affected in metastatic lymphadenopathy. To study the effectiveness of the cell block technique in morphological preservation of cells in metastatic lymph node lesions by comparison with conventional FNA sample. To determine the effectiveness of the cell block technique in preservation of immunohistochemical properties. To determine the role of immunohistochemistry in cell block preparations of malignant breast neoplasms in making a final diagnosis. To compare the results with other similar studies.

2. Materials and Methods

This study is conducted in the Department of Pathology, Government Medical College Srikakulam, from January 2022 to June 2023 for 18 months. The total sample size is 48. All the fine needle aspiration samples of clinically suspected cases of malignancy attending the cytopathology lab are considered as study material.

1. FNAC: The procedure is explained to the patient, and written consent is taken. The site of puncture is cleaned with a local antiseptic lotion, and a syringe with a 22-gauge needle is positioned in the tumor [2,14].
2. Cell block preparation with a mixture of 9 parts of ethyl alcohol and 1 part of 10% formalin and subjected to hematoxylin & eosin staining [3].
3. Immunohistochemistry staining technique.

2.1. Inclusion Criteria

Patients presenting with clinically suspected metastatic lesions, irrespective of sex, caste, religion, socio-economic status, and severity of illness.

2.2. Exclusion Criteria

FNAC samples of non-neoplastic lesions. Patients not willing for FNAC. Inadequate yield of FNAC samples. Patients on adjuvant or neoadjuvant chemotherapy. Patients who are in radiotherapy or post radiotherapy.

H&E staining: Firstly, the sections were deparaffinized in xylene and gradually hydrated through graded alcohol, i.e., 100, 95, 80, and 70% ethanol, respectively. Secondly, the sections were stained in hematoxylin solution and differentiated in 1% hydrochloric alcohol, then rinsed with tap water and distilled water until the nuclei become blue, then dehydrated in 95% ethanol. Thirdly, the sections were counterstained in 1% eosin solution, washed with 70% ethanol twice and absolute ethanol, and then cleared in 2 changes of xylene. Lastly, the sections were mounted with DPX mountant and observed under microscopy (MLX: magnus) [6].

2.3. Immunohistochemistry

Firstly, the sections were deparaffinized in xylene and gradually hydrated through graded alcohol to water. Secondly, the sections were immersed in H_2O_2 and heated in a microwave oven, washed with phosphate-buffered saline (PBS; pH 7.4), and immersed in citrate buffer solution (pH 6.0). Thirdly, the sections were blocked with nonimmune serum, stained with the primary antibody (AE1/AE3), and then with the secondary rabbit anti-mouse (HRP) IgG antibody (DAKO). The sections were incubated with SP (streptavidin-peroxidase) and then freshly prepared DAB solution for color development. Lastly, the sections were counterstained with hematoxylin, cleared in water, mounted with neutral balsam, and observed under microscopy (MLX: Magnus). Interpretation of the results: The results of pathological and immunohistochemical examinations were evaluated by 2 senior pathologists, who were not aware of the lymph nodes and the patients' diagnosis before the examination [4,6].

3. Results

Patients had an age range from 10 to 80 years, with the maximum cases observed in the old age group of 50 to 80 years. In this study, there were 38 male cases, resulting in a male-to-female ratio of 3.8:1.

The majority of malignancies were found in cervical lymph node aspirations, followed by axillary and inguinal lymph node aspirations (9 cases). The demographic distribution of cases is shown in Table 1.

The distribution of cases according to the site (total 48 cases) is presented in Table 2.

In the present study, pan CK positivity was found in 31 out of 48 cases. Among these, 25 were squamous cell carcinomas, 2 were suspicious for malignancy, and 2 were classified as other cases.

The distribution of cases according to H&E staining and cytokeratin typing is shown in Table 3.

Table 1. Demographic distribution of cases

Age in years	No of cases
10-30	5
30-50	9
50-70	18
70-80	16
Total	**48**

Table 2. Showing site wise distribution of cases (total 48 cases)

Site	No of cases
Cervical lymph node	27
Axillary lymph node	12
Ingiunal lymph node	9

Table 3. Distribution of cases according to H&E staining, cytokeratin typing

Name of lesion	**H&E staining**	**AE1,AE3 IHC**
Inflammatory	12	One case positive ,11 negative
Malignancy	36	
Adeno carcinoma	5	One weakly positive 4 negative
Squamous cell carcinoma	25	Strongly positive
Lymphoma	2	negative
Suspicious of malignancy	2	Strongly positive
Others	2	Strongly positive
Total number of cases	**48**	48

4. Discussion

The present study aimed to evaluate the importance of immunohistochemical expression of AE1/AE3 on FNAC cell blocks in diagnosing metastatic deposits in lymph nodes [13].

Lymph node metastasis is a common problem in patients, playing a crucial role in the clinical and pathological staging of malignancies [12].

Early diagnosis and intervention for lymph node metastasis are essential for effective treatment and prognosis of the disease [16]. In this study, the expression of AE1/AE3 was assessed.

Cytokeratin, an important tumor marker of epithelial origin, is used to study epithelial-originated neoplasms.

Cytokeratins are widely distributed intermediate filaments that play a key role in cell differentiation, proliferation, and nuclear changes. Normal lymph nodes lack an epithelial component, so the expression of cytokeratins indicates metastasis [16].

The cytoplasm and cell membrane of epithelium-derived carcinoma cells appeared brown and were easily identifiable.

We observed a higher number of cases in the age group of 50 to 80 years, and the male-to-female ratio was 3.8:1, which is comparable to the findings of Anand et al. [2].

Out of 48 aspirates, 36 were diagnosed as malignant based on cellular features of anaplasia and pleomorphism. Among the 12 inflammatory cases, 7 showed features of Tuberculosis with clusters of epithelioid cells, caseating necrosis, and giant cells.

Tuberculosis is prevalent in India and is a common cause of lymphadenopathy [9]. One case showed intense inflammation on H&E staining but was diagnosed as malignancy on IHC with positive AE1/AE3.

On H&E staining, 2 cases were diagnosed as suspicious of malignancy, and another 2 cases were diagnosed as poorly differentiated carcinoma, all of which showed positive AE1/AE3 on IHC.

This is the main advantage of IHC on cell blocks, as it allows us to confirm malignancy in cases of discrepancy without resorting to biopsy [8].

Out of 48 cases, 29 cases (60%) showed strong positivity for IHC, which is comparable to the findings of Anand et al. [2] who reported 66% positivity.

Among the 25 cases diagnosed as squamous cell carcinoma on H&E staining, all showed 100% strong positivity on IHC, comparable to the findings of Sandra J. Shin et al. [14] with 87.5% positivity.

We observed a higher number of malignancies in cervical lymph node aspirations compared to axillary and inguinal lymph node aspirations (9 cases). The most common malignancy observed was squamous cell carcinoma.

Oral cancers are more common in North Coastal Andhra Pradesh due to the prevalent habit of reverse smoking, in addition to tobacco chewing [11].

IHC techniques have been widely used to demonstrate various antigens and differentiate between malignant and benign diseases [2].

In the present study, strong positivity of AE1/AE3 was observed in 29 out of 48 cases. Among these, 23 were squamous cell carcinomas, 2 cases were suspicious for malignancy, and 2 cases were poorly differentiated carcinoma. IHC was negative in lymphoma cases, weakly positive in one case of adenocarcinoma, and negative in 4 cases of adenocarcinoma.

Pan-CK AE1/AE3 is useful for identifying squamous differentiation [15]. Pan ck AE1/AE3are used alog with panel EMA, CK7/CK20used for epithelial &adenocarcinoma differentiation in tumors and metastic lymphnodes [7,8,10].

5. Conclusion

The cytomorphological diagnosis of benign vs malignancy is enhanced by use of cell block & immunohistochemistry method. Many ancillary studies can be done by using cell blocks to increase the prediction of malignancy and even for prognostic information. The Pan CK (AE1/AE3) showed good percentage of positivity to identify squamous differentiation. The panel of markers along with can be used for epithelial differentiation. It helpful neoadjuvant therapy and patient care.

Author Contributions: All authors contributed equally to the writing of this paper. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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Appendix Images

Figure 1. H&E stain, 40x view showing pleomorphic epithelial cells with round to oval shape hyperchromatic nucleus and abundant eosinophilic cytoplasm

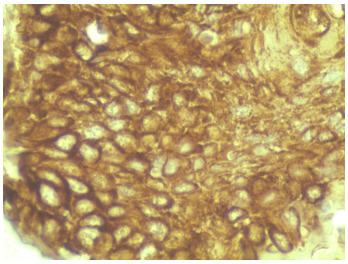

Figure 2. IHC40x view showing ckA1ckA3 stain showing cytoplasm positive

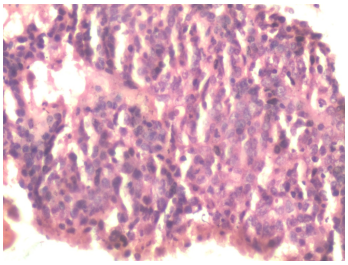

Figure 3. H&E stain: 20x view showing secondary deposits -squamous cell carcinoma shows sheets of pleomorphic cells

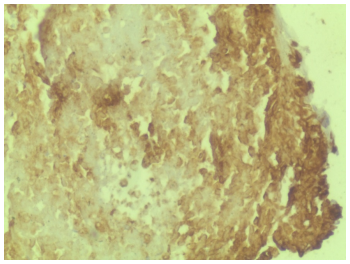

Figure 4. IHC, 20x power showing pan ck stain cytoplasm positive in secondary deposit from squamous cell carcinoma

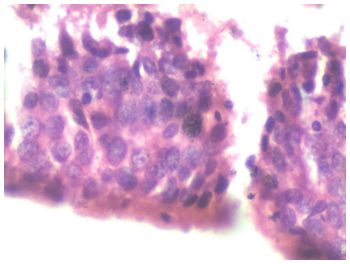

Figure 5. H&E stain: 40x view showing pleomorphic cells with round to oval shape hyperchromatic nucleus, focal areas showing granular chromatin scanty cytoplasm

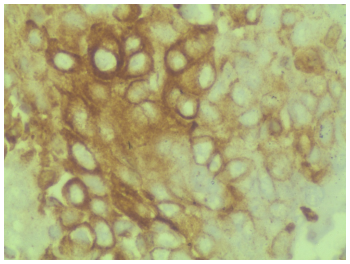

Figure 6. IHC, 40x view showing pan CK stains cytoplasm positive in secondary deposits from squamous cell carcinoma

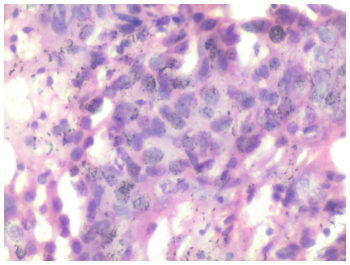

Figure 7. H&E: 40x view showing pleomorphic squamous cells showing hyperchromatic nuclei eosinophilic cytoplasm with high N/C ratio

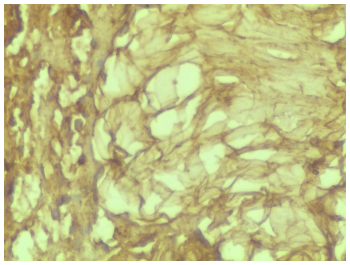

Figure 8. IHC: 40x view showing ckA1 ckA3 stain showing cytoplasm positive

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