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Role Of Immunohistochemistry In Metastatic Carcinoma Of Unknown Primary

[1]Dr. Sheikh Aamer Saleem, [2]Dr. Roopa Kodimyala
[1]M.D., Assistant Professor, [2]M.B.B.S., M.D, Senior Resident,
Department of Pathology, Kamineni Academy of Medical Sciences & Research Centre, Hyderabad, Telangana State, India

***Corresponding Author:**
Dr. Sheikh Aamer Saleem
Assistant Professor, Department of Pathology, Kamineni Academy of Medical Sciences & Research Centre, Hyderabad, Telangana state, India. Pin code – 500074

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Abstract

Background

Carcinoma of Unknown Primary Origin (CUP), a heterogeneous disease characterized by aggressive clinical course that is less responsive to chemotherapy. Initial work up should focus on identifying patients with favorable subgroups. Immunohistochemistry is a reliable and widely available tool in identification of primary origin.

Aim

To evaluate the role of Immunohistochemistry in diagnosing the metastatic carcinoma from unknown primary.

Materials And Methods

It is a retrospective study of 24 cases of metastatic carcinoma of unknown primary tumor, collected over period of 20 months from January 2019 to August 2020 .

Results

Out of 24 cases, after routine histopathological evaluation and immunohistochemistry panel application, primary could be identified in 22 cases and in 2 cases primary could not be identified.

Conclusion

In CUP cases, identification of subset of tumors with favorable features may have a longer disease free survival is an important step in management, where immunohistochemistry plays an important role. Routine histopathology and Immunohistochemistry along with clinico radiological correlation can solve the problem

Keywords: Immunohistochemistry, Carcinoma of Unknown Primary Origin

Introduction

Carcinoma of unknown primary (CUP) are metastatic carcinomas whose primary site of origin cannot not be ascertained despite a multidisciplinary approach which includes detailed medical history, clinical examination and diagnostic work up (1). Among all the malignant cases, about 2-5% of them are identified as malignancy with metastasis of unknown primary tumor origin(2). In view of targeted therapies for malignancies, the need to identify subsets of carcinoma of unknown primary, and warrants further histopathological examination with the help of immunohistochemistry [IHC] (3,4). It is a difficult challenge for the pathologist while dealing with the small biopsy specimens to determine or identify primary site of tumor origin. With judicious use of IHC based on morphology, about 90% of the undifferentiated malignant tumors could be diagnosed(5).

Subjects And Methods:

This is a retrospective study and analysis of medical records of all patients with metastatic carcinomatous lesions received in the department of pathology, Kamineni academy of medical sciences. The duration of study was 20 months from January 2019 to August 2020. A sample size of 24 cases was studied and in order to determine the primary tumor, the lesional sites were biopsied and sent for histopathological diagnosis. After proper fixation and tissue processing, 3-4 microns thick section of paraffin blocks were cut followed by staining with hematoxylin & eosin (H&E) and studied by two experienced pathologist. Based on subsequent morphological characteristics, the IHC markers were used to determine the primaries. IHC was performed manually on formalin fixed paraffin sections in cases of unknown primary. Various IHC markers used are Pan-CK (Cytokeratin), CK-7, CK-20, TTF-1 (Thyroid transcription factor -1), ER (Estrogen receptor), PR (Progesterone receptor), Chromogranin A, LCA (Leucocyte common antigen), WT-1 (Wilm's tumor), Calretinin, Gross cystic disease fluid protein (GCDFP15), CDX-2 (Caudal type Homeobox -2), CEA (carcinoembryonic antigen) & CD20 (Cluster of differentiation 20).

Results:

Among the 24 cases, most common metastatic site was bone (6 cases) constituting to about 25% and the second most common site was liver (5 cases) that counts to 20.8%. The most common age group affected were from 61-70 years (6 cases). The sex incidence in various histological types of secondary deposits is shown in table 1. All the cases presented with metastasis in single anatomical region. After applying IHC, initially with CK7 & CK20 for 23 cases and for one case LCA and Pan-CK was done.

CK7 +ve / CK20 –ve was seen in 21 cases, CK7 / CK20 both were negative in two cases and one case was LCA +ve. Among the CK7 +ve cases, most common metastatic histological tumor lesion was adenocarcinoma lung which are TTF-1 & CEA positive contributing to 29.1% of cases. CEA was also done in these cases to rule out any thyroid malignancies. Following them are 5 cases (20.8%) of metastatic duct cell carcinoma in which ER, PR, CEA& GCDFP was positive. Four cases (16.6%) of ovarian adenocarcinoma with WT-1 positive and CDX-2 negative in all of these four cases in which one of them had suprarenal metastatic site. Three cases (12.5%) were diagnosed as metastatic small cell neuroendocrine carcinoma of lung with chromogrannin A and TTF-1 were positive.

Among the miscellaneous cases which are CK7 +ve / CK20 -ve, a case of follicular thyroid carcinoma with thoracic vertebrae 11 as its metastatic site which is TTF-1 positive and CEA negative. Another case with liver as metastatic site, in which primary tumor site could not be found out. A single case of CK7 -ve / CK20 –ve with lung as metastatic site was positive for calretinin and WT-1 and diagnosed as mesothelioma, and in another case with CK7 & CK 20 both negative with left shoulder soft tissue mass as metatstaic site, primary tumor site could not be identified. A right sided axillary soft tissue lesion was diagnosed as Non-Hodgkin Lymphoma (LCA & CD20 positive). The various metastatic anatomical region along with histological tumor lesions are shown in table 2.

Discussion:

Histopathology is cornerstone in diagnostic procedure of CUP. A good biopsy specimen is of great importance, especially in cases of poorly differentiated carcinomas (6). The criteria for CUP include a biopsy proven malignancy for which the anatomic origin is unknown even after a medical history is being obtained, a detailed physical examination has being performed, liver & kidney function test, blood test & radiological being done (7).

IHC markers, usually peroxidase-labelled antibodies against specific tumor antigens, are helpful in determining the tumor lineage (8). Each and every individual IHC markers are different in nature as few are them are nuclear proteins, specific cellular products or few expressed on surface of cells etc and each IHC marker has different site of staining as of nuclear / cytoplasmic or both / membranous, hence it is important to know about them such that only the true staining is accepted (9).

There are twenty known subtypes of cytokeratins, which are intermediate filaments having different molecular weights each. The Cytokeratins help to classify tumors according to site of origin, with most commonly used in practice are CK7 & CK20. CK7 is expressed in cholangiocarcinoma, upper

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gastrointestinal tract, pancreas, ovary, lung, breast and endometrial tumors whereas CK20 is expressed in lower gastrointestinal tract lesions, urothelial tumors or Merkel cells (10).

TTF-1 is useful in determining thyroid and lung as primary site of tumor origin. The small cell neuroendocrine lung tumors are positive for TTF-1, CK7 (11). To confirm the neuroendocrine origin chromogrannin A is used which is a member of granin family of neuroendocrine secretory proteins.

ER and PR are nuclear proteins whose expression are restricted to breast, ovary and elsewhere in gynecological tract (12). CEA is an oncofetal glycoprotein expressed in colon, stomach, breast, pancreas and mucinous tumors of ovary (13). Wilm's tumor is also a nuclear protein which acts as archetypal tumor suppressor gene, as both alleles need to be deleted or inactivated for tumors to develop(14). It is expressed in wide range of tumors from epithelial, mesenchymal, haematopoietic & neural tissues (15). In our study, WT-1 helped in differentiating ovarian neoplasm with meaothelioma with the help of another IHC marker calretinin, as in mesothelioma calretinin is positive but not in ovarian neoplasm. Calretinin is a calcium binding protein of calmodulin superfamily which is widely used for mesothelial differentiation (16). CDX-2 is a homeo box protein transcription factor which is expressed in proximal duodenal epithelial cells to distal rectal epithelial cells (17). It helped in differentiating ovarian neoplasm from intestinal neoplasm, as in ovarian malignancies they are negative.

In the study conducted by Perry et. al investigated 68 cases of brain metastasis. The markers which were helpful in determining primary site of tumor origin were CK7, CK20, ER & GCDFP-15 (Gross cystic disease fluid protein-15). All these markers are used to differentiate between lung, breast, gastrointestinal or renal tumor origins(18). About 128 metastatic adenocarcinomas from five different sites (breast, colon, lung, ovary and upper gastrointestinal tract) were studied by Brown et.al and they tested eight markers and selected four CEA, CA199, CA125 & BCA225 (breast cancer antigen 225) (19) .

In the study, conducted by Roy et.al, the most common site for metatstasis was lung (23.6%) and in our study it was bone (20). An observation made by Bohinski et.al was that none of the pulmonary lesions showed a positive result with TTF-1 (21). A good knowledge of morphological features of the initial hematoxylin & eosin stained sections determine the use of various IHC markers and a protocol to be set to investigate each case

Tables:

Table 1 : **Sex incidence of various histological types of metastatic deposits**

Sex	Small cell neuroendocrine lung carcinoma	Adenocarcinoma lung	Adenocarcinoma ovary	Adenocarcinoma breast	Miscellaneous	Total
Male	2	7	-	-	3	12
Female	1	-	4	5	2	12
Total	3	7	4	5	5	24

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Table 2: Incidence of specific histological types in different metastatic sites

Metastatic sites	Adenocarcinoma	Small Cell Neuroendocrine carcinoma-lung	Non-Hodgkin Lymphoma	Mesothelioma	Follicular carcinoma of thyroid	Metastatic carcinoma – primary site not found	Total
Axillary node	1	1					2
Cervical node	2						2
Liver	2	2				1	5
Bone	5				1		6
Lung	2			1			3
Omentum	3						3
Soft tissue			1			1	2
Suprarenal mass	1						1
Total	16	3	1	1	1	2	24

Figures:

FIG 1: Poorly differentiated carcinoma in cervical lymph node, H and E (40x)

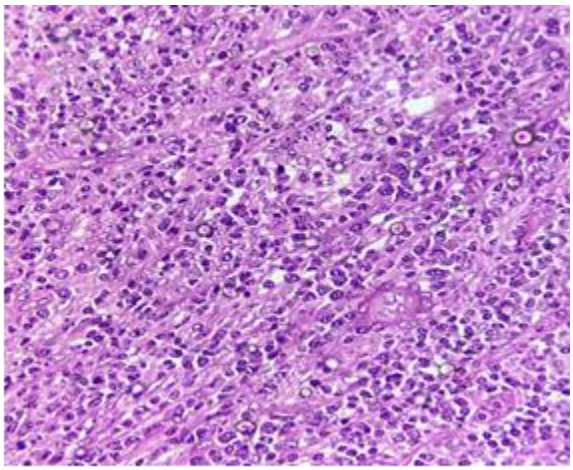

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FIG 2: Immunohistochemistry staining of CK 7 positive poorly differentiated carcinoma cervical lymph node (40x)

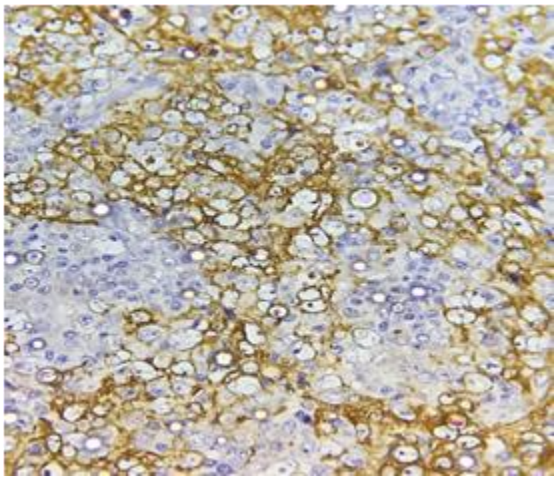


FIG 3: Metastatic adenocarcinoma of bone, H&E (40x).

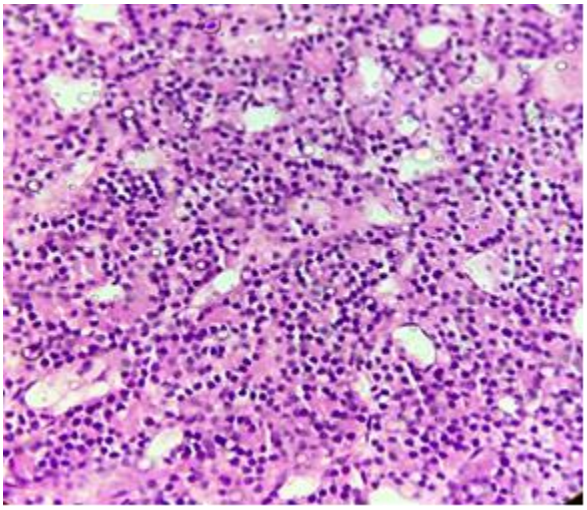
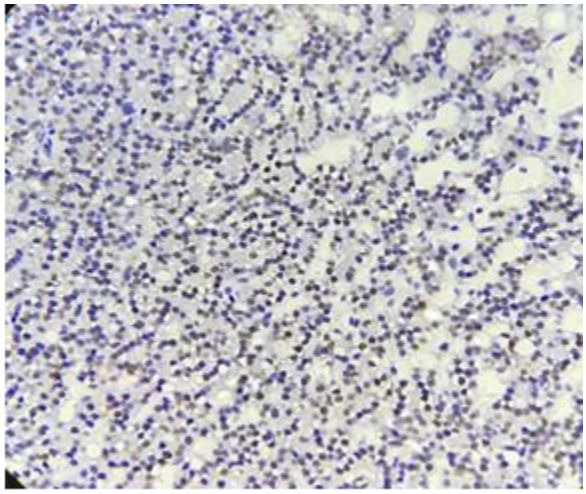


FIG 4: Immunohistochemistry staining of TTF-1 positive in metastatic adenocarcinoma of bone (40x)



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