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AN OBSERVATIONAL STUDY OF ROLE OF IMMUNOHISTOCHEMISTRY IN DIAGNOSING ORIGIN OF METASTATIC CARCINOMAS WITH UNKNOWN PRIMARY SITE.

Pathology

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ABSTRACT

Background & objectives: Carcinoma of unknown primary (CUP) is a biopsy proven malignancy for which the anatomic site of origin remains unidentified after a focused search. IHC may be useful for determination of primary site as identification of origin affects prognosis and treatment in the era of novel targeted therapies. The purpose of study is to find out the primary site of tumor with application of diagnostic and predictive markers so that diagnosis and proper treatment can be started in these patients. And to find out number of CUP lesions in these cases. As these cases are managed by special therapeutic regimes. **Methods:** A Cross-sectional study including 42 patients diagnosed as metastatic carcinoma with unknown primary site referred to Department of Pathology, SMS Medical college and hospitals, Jaipur (Rajasthan) in year 2021-2022. Histopathological Examination after staining with Hematoxylin and Eosin (H &E) stain And Immunohistochemical staining on formalin-fixed paraffin -embedded tissue blocks were carried out. **Results:** Out of 42 cases, The age group (40-60) years have highest percentage (50%)with male predominance (66.7%) with male: female 2:1. Lymph node represents most common site of presentation of metastatic deposits (31%) followed by liver (24%), bone (21%), lung (7%), brain (3%). Others (14%). Adenocarcinoma (54.8%) was the most common histological variants followed by undifferentiated neoplasms(16.7%), Squamous cell carcinoma(9.5%), Carcinoma with neuroendocrine origin (9.5%). Lung(26.2%) was the most common primary site followed by Gastrointestinal tract (16.7%) In 28.6% cases the primary origin remain unknown even after extensive immunohistochemistry. CK7+/CK20-(69%) pattern of expression was the most common pattern followed by CK7-/CK20- (16.7%) , CK7+/CK20+ (11.9%) and CK7-/CK20+ (2.4%). **Interpretation & conclusion:** IHC study may aid in the diagnosis of CUP and is very important in the era of targeted therapy which indeed can improve the survival rate of patients. This would impact patients prognosis and management allowing site- specific therapy. Prognosis for the patients is still poor due to late presentation and need of extensive workup to find out primary resulting in delayed treatment. However ,some cases still remain unknown origin after IHC study, so more diagnostic methods and gene expression studies may be needed for definitive diagnosis.

KEYWORDS

Carcinoma of unknown primary, Immunohistochemistry, Cytokeratins,

INTRODUCTION:

Cancer is a formidable challenge for physicians, scientists, epidemiologists, healthcare providers, and most importantly, for patients and their families. Worldwide, an estimated 19.3 million new cancer cases and almost 10.0 million cancer deaths occurred in 2020.[1] Cancer Metastasis is the primary cause of morbidity and mortality and responsible for about 90% of cancer deaths. .[2]

Metastatic Carcinoma of unknown primary (CUP) is a biopsy proven malignancy for which the anatomic site of origin remains unidentified after a focused search. Metastatic malignancy is one of the 10 most frequently diagnosed cancers worldwide[3], accounting for 3–5% of all human cancers, reported to be the seventh to eighth most frequent malignant tumor, and is the fourth most common cause of cancer death in both sexes. Median age at presentation is 65–90 years. The disorder is slightly more common in men than in women, and predominantly affects adults[5] The overexpression of various genes, including ras, bcl-2, her-2 and p53 has been identified in malignant carcinoma samples.[4] CUP is clinically characterized as an aggressive disease with early dissemination. Diagnostic approaches to identify the primary site include detailed histopathological examination with specific immunohistochemistry and radiological assessment.[5]

Metastatic adenocarcinoma is the most common CUP histopathology (80%). Patients presenting with metastasis are divided into subsets of favourable (20%) and unfavourable (80%) prognosis. Favourable subsets are mostly given locoregional treatment or systemic platinum-based chemotherapy. Responses and survival are similar to those of patients with relevant known primary tumors.[5] The transmembrane proteins epidermal growth factor receptor, platelet-derived growth factor receptor, MET, and c-Kit, which contribute to malignant transformation.[5,6] The guardian of the genome, TP53, has been investigated in studies of CUP.[7]

Most cases of CUP are carcinomas, which are divided into adenocarcinomas of well or moderate differentiation (60%), undifferentiated or poorly differentiated adeno carcinomas (30%), squamous-cell carcinomas (5%), and undifferentiated neoplasms (5%). Immunohistochemical studies can help to categorize patients into subsets to receive the appropriate therapeutic management.[5]

For diagnosing primary site in metastatic malignancies, a detailed pathological examination and evaluation of most accessible biopsy tissue specimen is done using Hematoxylin and eosin stain and Immunohistochemistry (IHC). Advanced techniques i.e., FISH Molecular gene profiling is required in some cases.[8] Thorough clinical details and examination and investigations is essential to search for the primary tumor based on pathological evaluation of the metastases and determine the extent of disease..[4]

Histologic subtyping with the help of immunohistochemical characterization of tumors has resulted in diagnoses which tumor which was not previously possible. [4] Through the identification of specific cellular components of cell patterns, using a specific panel of monoclonal or polyclonal antibodies, the immunohistochemical method has transformed the diagnosis of these tumors. For treatment purposes, it is important to know whether an undifferentiated tumor is epithelial, mesenchymal or hematopoietic[8]

The purpose of study is to find out the primary site of tumor with application of diagnostic and predictive markers so that diagnosis and proper treatment can be started in these patients. And to find out

number of CUP lesions in these cases. As these cases are managed by special therapeutic regimes.

The primary objectives were to find out the most common site / organ affected by metastatic carcinoma. And to find out the most common site of unknown primary tumor with help of Immunohistochemistry. Study also aimed to identify which IHC panel detects the different tumor lineage and the role of radiology in minimizing the number of IHC panel.

MATERIAL AND METHODS:

In this Cross sectional Observational study from march 2021 to June 2022 ,the H&E -Stained slides archieved for the cases which presented with metastatic carcinoma for which primary site remained undetermined at the time of presentation .Immunohistochemistry is applied in this cases with the knowledge of demographic information including age, gender, health status, complete radiological details (Ct scan/ MRI scan/PET scan) along with serology (if done).

A stepwise approach was carried out for workup CUP cases in SMS Medical college and hospital, Jaipur .The biopsy obtained with proper site have been noted , the presence of metastasis has been confirmed on H&E sections .After that , IHC markers panels was applied in a stepwise approach to identify the broad tumor type , tumor subtype and further recognition of primary organ.

The study of 42 cases of metastatic CUP has been approved by Ethical committee of SMS Medical college ,Jaipur. The correlation of clinical information with application of cytokeratins and organ specific markers used in diagnosing primary site . Cytokeratins CK7 and CK20 were used primarily in all the cases . Depending on the expression of these two markers further antibodies were applied to reach the final diagnosis.

IHC was performed both manually and by Autostainer. Staining was performed by preparing 2-m thick sections from formalin-fixed paraffin -embedded tissue blocks and were dried in a 60^{0}C oven overnight. Then the sections were placed in a BOND-MAX Automated immunohistochemistry vision (Leica Microsystems GmbH, Wetzlar, Germany).

RESULTS:

Among 87 cases presented with metastasis during this period ,42 cases were studied which are having complete clinical details. In this study, 50%were found in age group 40-60 years, followed by 42.8% in age group 60-80 years.

The average age of the cohort group was found to be 58.2 years. Male patients were 29(66.7%) while Female patients found were 13(33.3%) with male : female to be 2:1.

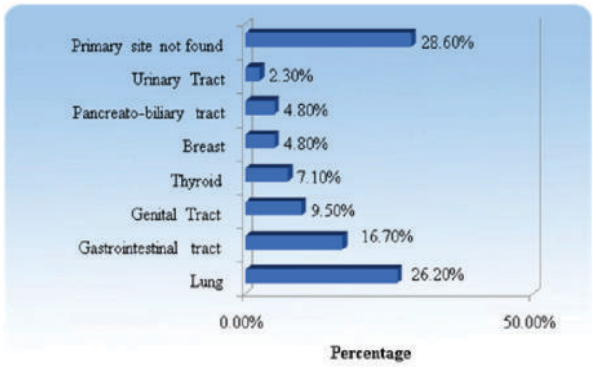
Lymph node was the most common site of presentation of metastatic deposits 13 (31%) followed by liver 10 (24%), bone 9 (21%), lung 3 (7%), brain 1 (3%). Other site (14%) includes nasopharynx, suprarenal mass, muscular swelling etc. Out of 13 lymph nodes, cervical lymph nodes involvement was most common followed by supraclavicular lymph nodes

The Histologic types identified in the studied cases were Adenocarcinoma 23 (54.8%) the most common histological variants among the metastatic carcinomas followed by undifferentiated neoplasms in 7(16.7%), Squamous cell carcinoma in 4(9.5%), Carcinoma with neuroendocrine origin in 4(9.5%) and other variants in 4 (9.5%).

Table : Histological Variants

S. No	Type	No. of cases	%
1	Adenocarcinoma	23	54.8%
2	Undifferentiated carcinoma	7	16.7%
3	Squamous cell carcinoma	4	9.5%
4	Carcinoma with Neuroendocrine Origin	4	9.5%
5	Others	4	9.5%

Lung was found out to be most common primary site with 11 cases (26.2%) followed by Gastrointestinal tract with 9 cases (16.7%), Genital tract with 4 cases (9.5%) .12 cases (28.6%) the primary was not found even after extensive immunohistochemistry. (Graph 1)



Graph 2: Primary Site on Basis of Immunohistochemistry

Among 42 cases all (100%) patients have done with CT Scan (Thorax and/or Abdomen) Out of 42, in 36 cases (85%) CT scan played key role in deciding immunohistochemistry markers judiciously while in 6 cases (15%) even CT scan could not guide to rule out primary site. MRI done in only few patients. 17 patients (40%) among 42 cases, have underwent Positron emission tomography (PET Scan). Out of these17 cases, PET Scan aided in diagnosis of 8 (48%) cases which were also confirmed by immunohistochemistry. In rest 9(52%) the primary remain unknown by PET scan and in few ruled out by immunohistochemistry.

Monoclonal antibodies to specific cytokeratins (ck) subtypes are considered as it helps to classify tumor according to their site of origin. Most commonly used ck stains are CK7 and CK20.On applying IHC, In this study CK7+/CK20-29 cases (69%) pattern of expression was the most common pattern in metastatic carcinomas with unknown primary origin followed by CK7-/CK20- 7(16.7%) as the second most common pattern of expression, CK7+/CK20+ 5(11.9%) and Ck7/ CK20+ 1(2.4%) came out to be the least common pattern.

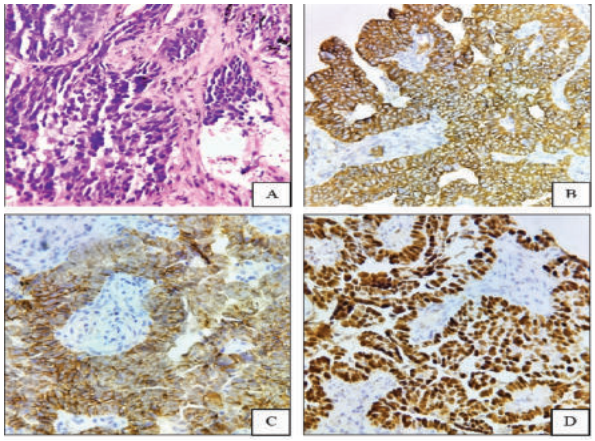


Figure 1: Liver Biopsy with Metastatic Mixed Mullerian Tumor. (A) H&E (B) PANCK+ (C) E CADHERIN+ (D) P53 + (Not shown CK7+, CK20-, GATA-3+, P16-, ER -, HER2 -.) (Magnification 40 x)

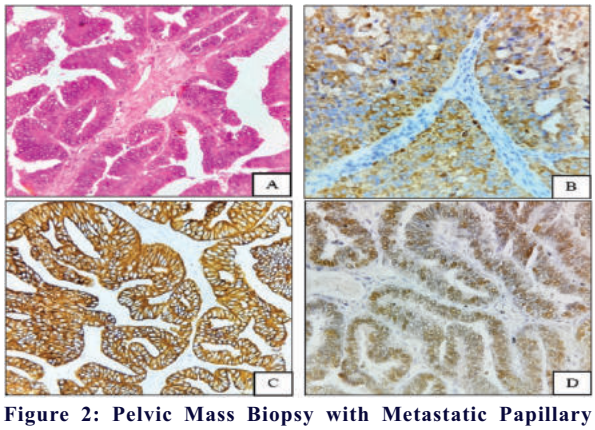


Figure 2: Pelvic Mass Biopsy with Metastatic Papillary

Adenocarcinoma of Pancreas (A) H/E (B) CK7 (C) CK19 + (D) P53+, (Not shown CK20-, WT 1-, PAX-8-. CDX-2-,Villin -,GATA 3-) (Magnification 40x).

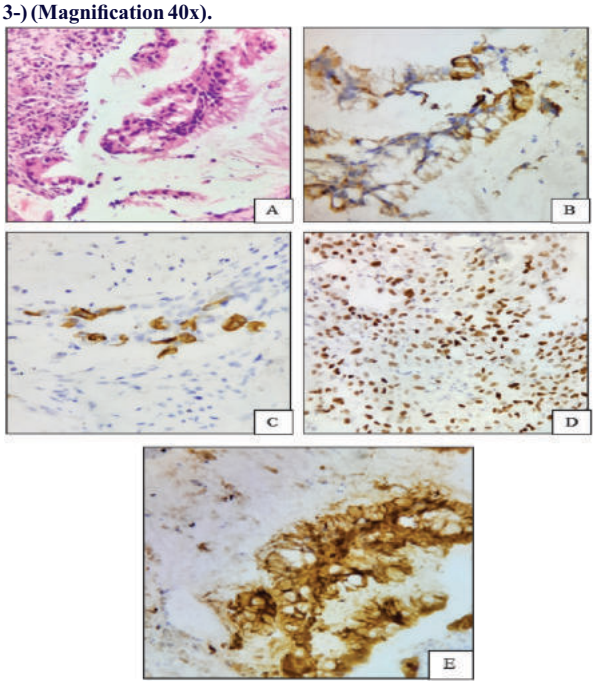


Figure 3: Scapular region biopsy with Metastatic pulmonary enteric Adenocarcinoma From Lung (A) H/E (B) Ck7 +(C) CK 20 + (D) TTF 1 + (E) Villin+ (Magnification 40x)

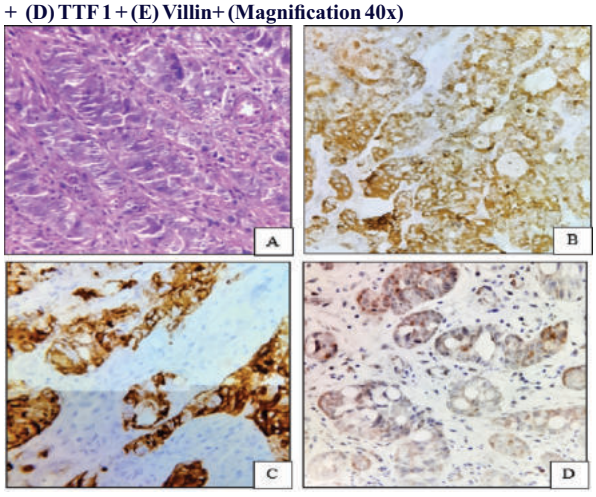


Figure 4: Vertebral Bone Biopsy with Metastatic Colorectal Adenocarcinoma. (A) H/E (B) CDX 2+ (C) CEA++ (D) Villin++. (Not Shown CK 20+, CK7 -,CK19-, TTF1-, Napsin A -) (Magnification 40x)

DISCUSSION:

Metastatic carcinomas are the cases where patient present with metastatic disease, but the primary site cannot be found .Identification of origin these lesions are highly important as it affects the prognosis and treatment, especially in targeted therapy.

Thorough histological examination on hematoxylin and eosin stain under light microscope is cornerstone in diagnosis. The wise use of ideal IHC markers (antibodies) correlating with clinical and radiological details is of great role in identifying origin of metastasis.

Among the numerous cases of metastatic malignancy from various sites were collected .out of which 42 cases are included in study (sample size n=42) during the study period which all have complete clinical and radiological details available.

IHC provides diagnostic guidance in approximately 90% of undifferentiated malignant tumors but usually at the end of a fastidious and expensive algorithm based on both morphology and IHC. However, identification of the primary site of origin may represent a difficult challenge for the pathologist when dealing with a small sample size along with increased generation of tumor-specific primary antibodies and the need complementary molecular analysis. If morphology is not sufficient, in particular for an undifferentiated neoplasm with no clear lineage differentiation, the first diagnostic IHC panel will include a few antibodies directed against epithelial antigens.[1]Two antibody classes are used in CUP work-ups: (A) Antibody to keratin (B) Antibody to organ restricted marker.[9]

Subclassification of carcinomas by high and low molecular weight keratins is useful, it is largely replaced by two more powerful discriminators, which are CK7 and CK20. There are four different patterns of expression including CK7+/ CK20+, CK7+/ CK20-, CK7-/ CK20+, and CK7-/ CK20-. These two important markers are used as the first step for the evaluation of carcinomas have limited capability in the definite diagnosis. These markers sometimes overlaps between the patterns Organ-specific makers of carcinomas are subdivided into two classes: (A) Cytoplasmic and/or membranous. Considering the fraction of tumor cells, which are positive for these markers, we can determine the state of differentiation of the tumor. (B) Nuclear transcription factors which are positive in all tumor cells and cannot determine the differentiation of the tumor.[9,10]

In this study, The average age of patients presenting with metastatic carcinomas was 58.2 years with male prepondrance (Male : Female) 2:1. The similar demographic observation was concluded in the studies of , Sharma P. et al. (2014)[14]. , and Omar E. et al. (2021)[16].

In present study ,lymph node was the most common site for metastatic deposits (31%) followed by liver (24%) ,bone (21%), lung (7%)and others (14%).Similar results were obtained in the studies conducted by Pentheroudakis G. et al.(2007)[12] . Lymph node was most common site of presentation in the studies of Omer E. et al. (2021)[16]. The study is in discordance with the study of Lazardis G.et al.(2008)[13] and Mokhtari M. et al.(2022)[9] where liver was the most common site of metastatic deposits may be due to specific anatomic site based study.

In this study, the most diagnosed histologic variant was adenocarcinoma 54.8% cases which was in concordance with the study of Penthroudakis G. et al. (2007)[60] , Lazardis G et al. (2008)[61] and Omar E et al. (2021)[66] revealing adenocarcinoma as most common histology. The proportion of other histologic variants were different due to heterogenous variability of metastatic malignancies.

In this study on applying immunohistochemistry, the most common primary site found was lung (26.2 %) followed by gastrointestinal and pancreticobiliary tract (21.5%), genital tract (9.5%). This finding is consistent with study of Penthroudakis G. et al. (2007)[12]. The observation in studies of Sharma P. et al. (2014)[14] and Roy S. et al. (2016)[15] are different may be due to anatomic site specific studies.

In our study CT Scan was found to be superior than PET Scan. In 85% of cases CT Scan played important diagnostic aid to carry out appropriated and judicious use of immunohistochemistry markers. As not easily affordable, PET Scan has been tracked out in 17 cases (40%) out of which in 48% the diagnosis by IHC was justified by PET Scan and vice versa**.** Similar literature has been reviewed by Seve P.et al. (2007).[11]

In our study, CK7+/CK20- pattern of expression was the most common (69%) followed by second most common CK7-/CK20 (16.7%), CK7+/CK20+ (11.9%) and least common CK7-/CK20+ (2.4%).Similar results were obtained in the studies conducted by Roy S.et al.(2016)[15] and Mokhtari M. et al. (2022)[9]. Although variations in internal proportion have been observed that may be due to accuracy of the markers and variability in expression of CK7.

Among the 29 **CK7+/CK20-**cases, 6 cases were of metastatic carcinoma of lung. TTF-1 and Napsin-A were applied. Out of 6 cases TTF-1 found to be positive in 5 cases and Napsin - A positivity was found in 4 cases.

Three cases were of Genital tract .One case was of metastatic mixed Mullerian tumor (PR+,GATA-3+, E-Cadherin+, ER-, HER2-, P16-).**(figure:1)** One of metastatic serous adenocarcinoma ovary (WT1+, PAX8+, PanCK +, CDX2-, P53-) and one as metastatic carcinoma of embryonal origin (ER, PAX-8 +,WT1-, P53-). These cases are further

correlated clinically and radiologically.

Three cases of metastatic carcinoma of thyroid (TTF-1+, PAX-8+). 3 cases were metastatic small cell (Neuroendocrine) carcinoma of lung (TTF-1+, PanCK+ and Synaptophysin+) among one of them showing Napsin -A positivity.Two cases were metastatic adenocarcinoma of Pancretico-biliary tract (CK19+, P53+, also applied ER, WT1, PAX8, CDX2, PR, Villin, GATA-3,TTF1, Napsin-A, Synatophysin, CD56 all of which found negative).**(figure :2)** Two cases (5%) were of metastatic adenocarcinoma carcinoma of breast (Her-2, EMA, GATA-3+, PAX-8+, TTF1-, Napsin A-, CDX-2-, ER-, PR-) breast. 6 cases (14%) among CK7+/CK20- primary remain undetected even after applying the organ specific antibodies.

Out of 5 cases of **CK7+/CK20+**, 3 (60% are metastatic adenocarcinoma from Gastrointestinal tract (CDX2, Villin+, CK19+, TTF1-, Napsin A-). Two (40%) of the 5 were case of pulmonary enteric adenocarcinoma (TTF1+, CDX2+, Villin+, Napsin A-). **(figure :3)**

CK7-/CK20+ is the least common expression with only 1 case (2%) of metastatic adenocarcinoma of gastrointestinal tract (CDX2+, Villin+, CEA+, TTF1-, Napsin A-) positive considered to be originated from colorectal region **.(figure:4)**

CK7-/CK20- are poorly differentiated carcinoma .one case found to be of gential tract (OCT ¾, PLAP, Vimentin, CD30+, Pan CK+, S100-, CDX 2-). Rest 6 cases are of metastatic adenocarcinoma with unknown primary origin .

Total 12 cases of 42 cases, primary site could not be identified on applying extensive organ specific antibodies.

Among the 12 cases where primary origin of metastatic carcinoma remain unknown, 6 cases expressed CK7+/CK20- while 6 cases expressed CK7-/CK20-. Metastatic adenocarcinoma with unknown primary predominantly expressed CK7+/CK20-(GATA-3 -, CDX-2-, TTF1-, Napsin A-, PAX-8-).

While among CK 7-/CK20- there are different histological varieties i.e. metastatic squamous cell carcinoma (P63+, CK5/6+, P40+ rest all organ specific markers came out to be negative.), neuroendocrine histology (Synaptophysin+) and undifferentiated carcinomas (EMA+, Vimentin+, Pan CK-, LCA-, WT1-, TTF1-, OCT3/4-, PLAP-, CDX 2-, MUM 1-, HepPar 1-, Glypican-, Inhibin-, CD15-, CD10-, PAX-8-).

Present study shows that in 71.4 % primary site of origin of metastatic carcinoma was detected while 28.6 % primary origin remains unknown. The study of Omar E. et al. (2021)[16] 67.3%, and Mokhtari M. et al. (2022)[9] 73% also have similar rate of detection of primary site. The study was not consistent with the study of Sharma P. et al. (2014)[14] and Roy S. et al. (2016)[15] with primary detection 86.84%, 85.5% respectively may be because of use of highly sensitive and specific newer markers.

CONCLUSION:

Metastatic carcinoma are a heterogenous group of malignancies with unambiguous biology. The subclassification of the metastatic tumor and accurate diagnosis of primary site after conventional correlation of radiological details with IHC is very important in the era of targeted therapy which indeed can improve the survival rate of patients. This would impact patients prognosis and management allowing site-specific therapy.

IHC is a composite diagnostic procedure by which a stepwise approach can help to detect origin of tumor primary site with specificity. Prognosis for the patients of metastatic carcinoma with unknown primary is still poor due to late presentation and need of extensive workup to find out primary resulting in delayed treatment.

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