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ORIGINAL ARTICLE

Adenocarcinoma with Unknown Primary: Diagnostic Implications using Immunohistochemistry

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ABSTRACT

Carcinoma of unknown primary site (CUP) is a heterogeneous group of cancers defined by the presence of metastatic disease with no identifiable primary tumor at presentation. We undertook this study to assess the utility of immunohistochemistry (IHC) for the determination of primary tumor site in adenocarcinomas. This retrospective study included 51 cases with a morphological diagnosis of metastatic adenocarcinoma with unknown primary. Basic IHC panel that included cytokeratin 7 (CK-7), cytokeratin 20 (CK-20), pan-cytokeratin (pan-CK), thyroid transcription factor-1 (TTF-1), and caudal type homebox 2 (CDX-2) was used. Additional extended panel with specific IHC markers was used in cases where the primary could not be determined using the basic panel. The male-to-female ratio was 1.4:1 with mean age of 50 years. The most common metastatic site was lymph node followed by liver. A conclusive diagnosis using IHC was achieved in 30 cases (58.82%). Specific diagnosis could be made in 8 cases (16%) using basic IHC panel.

Extended panel yielded specific diagnosis in additional 22 cases (43.13%). Primary site could not be determined using even both the panels in 21 cases (41.18%). The panels for identification of the primary need to be flexible depending on the site of metastasis, age/sex of the patient, and detailed history, which may determine sensitivity and specificity of primary detection.

Keywords: Adenocarcinoma, Immunohistochemistry, Neoplasms, Unknown primary.

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INTRODUCTION

Carcinomas of unknown primary sites are defined as a heterogeneous group with metastatic disease for which the site of origin cannot be identified at the time of diagnosis despite careful clinical examination and laboratory test.[1] The CUPs account for approximately 3% of all malignant neoplasms while adenocarcinoma accounts for about 50% of all CUPs. In this era of personalized medicine with targeted therapy being available, it is essential for a pathologist to identify the organ of origin, as it has a significant impact on overall survival.[1,2]

The role of a pathologist in identifying the organ of origin in cases of CUPs is expanding. Recent advances in IHC and the development of numerous organ-specific IHC antibodies have been of great help in this regard.[2,3] Current study was undertaken with the objective to identify the primary tumor site in cases of CUPs with adenocarcinoma phenotype.

MATERIALS AND METHODS

This was a retrospective tertiary care center, hospital-based study that included 51 cases diagnosed as metastatic adenocarcinoma in which the primary site of tumor was unknown. Testing with A panel of IHC markers was performed that was categorized into two groups: (1) basic IHC panel and (2) extended IHC panel (Table 1).

Immunohistochemistry testing was done by the standard protocol. The tissue section on coated slides was fixed overnight at 60°C in a dry oven, deparaffinized in xylene and rehydrated through graded ethanol series. Sections were blocked with 3% hydrogen peroxide in methanol for 30 minutes to quench any endogenous peroxidase activity, if present, and were then processed for antigen retrieval in Pascal (DAKO Cytomation, California) by placing in sodium citrate buffer (pH-6.0). Sections were incubated for an hour with primary antibodies, followed by treatment with polymer-based secondary antibody kit (Dakopatts, Envision kit, Denmark). Bound antibody was visualized using diaminobenzidine, according to the manufacturer's instructions. Sections were counterstained with hematoxylin and mounted. Positive and negative controls were run with all batches by including and omitting primary antibodies respectively.

The IHC results were interpreted under light microscopy. The results were analyzed in terms of the utility of the basic panel and the extended IHC panel for the identification of primary site in cases of CUPs with adenocarcinoma phenotype.

RESULTS

This retrospective tertiary care center, hospital-based study included 51 cases of metastatic adenocarcinoma in

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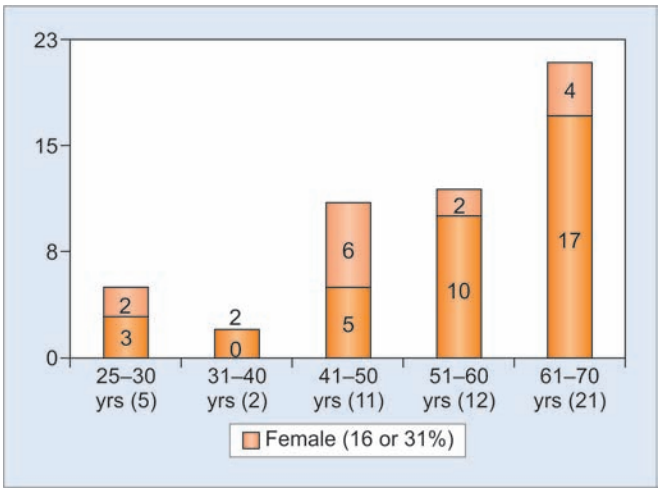
Table 1: List of IHC antibodies used for identification of primary tumor site

Name	*Clone*	*Supplier*	*Dilution*
Basic IHC panel			
Pan-CK	AE1/AE3	Dako	Ready to use
CK-7	OV-TL 12/30	Dako	Ready to use
CK-20	Ks20.8	Dako	Ready to use
TTF-1	8G7G3/1	Dako	Ready to use
CDX-2	DAK-CDX2	Dako	Ready to use
Extended IHC panel			
Thyroglobulin	DAK-Tg6	Dako	1:100
WT-1	6F-H2	Dako	Ready to use
Vimentin	V9	Dako	Ready to use
Synaptophysin	DAK-SYNAP	Dako	Ready to use
Epithelial membrane antigen (EMA)	E29	Dako	Ready to use
ER	EP1	Ventana Medical Systems	Ready to use
PSA	Anti-PSA	Dako	Ready to use
CK-5/6	D5/16 B4	Dako	Ready to use
Placental alkaline phosphatase (PlAP)	8A9	Dako	Ready to use
Carcinoembryonic antigen (CEA)	II-7	Dako	Ready to use
CD-34	QBEnd10	Dako	Ready to use
Hepatocellular carcinoma antigen (HCC-Ag)	OCH1E5	Dako	Ready to use
p-63	4A4	Biogenix	Ready to use
S-100	Anti-S-100	Dako	Ready to use
CD-10	56C6	Dako	Ready to use
Glial fibrillary acidic protein (GFAP)	Anti-GFAP	Dako	Ready to use
GCDFP	Anti-GCDFP	Dako	Ready to use
Leukocyte common antigen (LCA)	2B11 + PD7/26	Dako	Ready to use



Graph 1: The age and gender distribution

which the primary site of tumor was unknown. The male-to-female ratio was 1.4:1. The mean age of the patients in the study was 50 years (Graph 1).

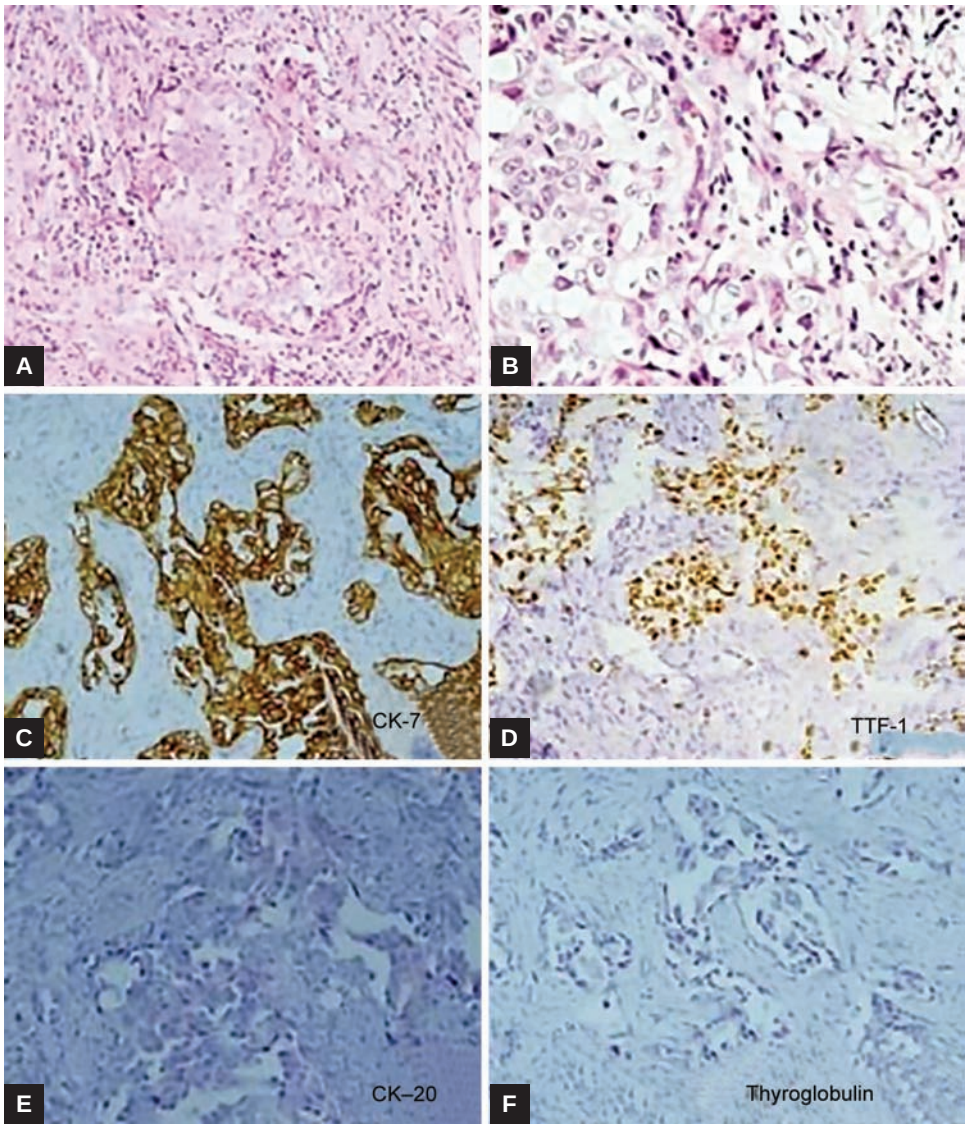
The most common metastatic site was lymph node followed by liver. A conclusive diagnosis using IHC was achieved in 30 cases (58.82%). The most common primary site was lung in 46.67% (n = 14), followed by the gastrointestinal tract (GIT) in 23.33% (n =7), thyroid in 10% (n = 3), breast in 6.67% (n = 2), ovary (n = 2) in 6.67%, prostate (n = 1) in 3.33%, and kidney (n = 1) in 3.33% (Figs 1 to 3).

The basic IHC panel constituted of CK-7, CK-20, TTF-1, CDX-2, and pan-CK. Specific diagnosis using this basic panel could be made in 8 cases (15.68%). Primary site could be identified with the extended panel in an additional 22 cases (43.13%). Primary site could not be determined using even both the panels in 21 cases (41.18%).

DISCUSSION

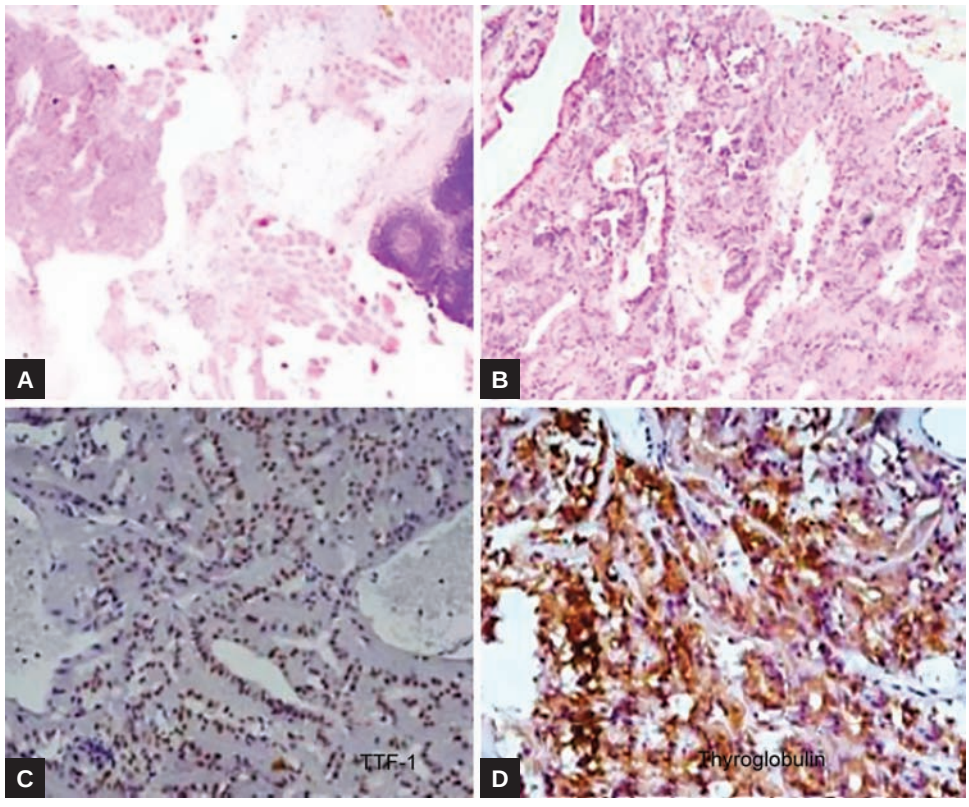
In this study, the utility of IHC panel in the diagnosis of primary tumor site in metastatic adenocarcinoma has been evaluated and validated. Through recent advancements in IHC, additional organ-specific antibodies have become available including estrogen receptors (ER), mammaglobin, gross cystic disease fluid protein-15 (GCDFP-15), CDX-2, TTF-1, Wilms' tumor susceptibility gene 1 (WT-1), paired box gene 8, prostate-specific antigen (PSA), and uroplakin with conventional antibodies including CK-7 and CK-20. Use of these biomarkers has the potential to identify the primary tumor site with greater sensitivity and specificity.[1,2] Improving treatment of CUPs requires identification of the primary tumor site using molecular markers and application of primary tumor site-specific treatment.[4]

In this study, two panels for IHC were designed and staining was performed in a sequential pattern. The basic panel was done initially to narrow down the

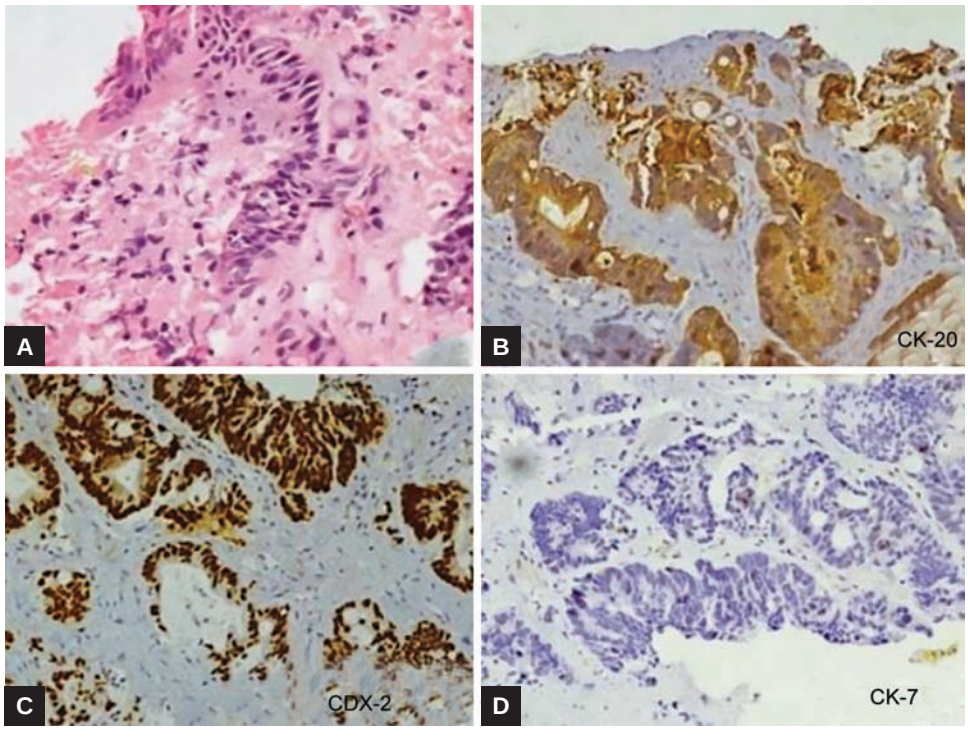


Figs 1A to F: (A and B) Cervical lymph node biopsy with tumor cells arranged in nests and acini (hematoxylin and eosin, A = ×100, B = ×200) with positivity for CK-7 (C) and TTF-1 (D) and negative staining for CK-20 (E) and thyroglobulin (F) indicative of primary in the lung (CK-7 = 3,3′-Diaminobenzidine (DAB) ×100, TTF-1 = DAB ×100, CK-20 = DAB ×100, thyroglobulin = DAB × 100)

probability of the primary site by using CK-7 and CK-20. The common CK-7-positive tumors are lung, thyroid, breast, ovary, upper GIT, and pancreaticobiliary tract, while CK-20 expression is commonly present in lower GIT along with urothelium, though it can be identified heterogeneously in other sites also. The primary site could be determined in 15.68% of cases using the basic panel. In the remaining cases, an extended panel was performed that included more organ-specific antibodies. An accurate diagnosis suggestive of the primary site could be achieved in additional 43.13% of cases using the extended panel. The overall diagnostic efficacy of IHC in the diagnosis of primary tumor sites in cases of CUPs with adenocarcinoma phenotype was 58.82% in the present study. In the study conducted by Hashimoto et al, the diagnostic efficacy was 81.7%.[1,5,6] The use of sequential IHC is important, as it aids in saving both time and resources. Utilization of an extensive panel of IHC in the beginning may lead to wastage of resources and may also cause exhaustion of the tissue biopsy. It is essential to treat all biopsies as precious samples, as molecular diagnosis is essential for the use of targeted therapy.[7] The College of American Pathologists recommends testing for actionable targets in multiple cancers like lung, colon, breast, stomach, and thyroid. Hence, it is essential to perform a robust panel of IHC for the detection of primary site in cases of CUPs but in a sequential pattern. Identifying patients with prognostically favorable disease is important in cases of CUPs, as they may get substantial benefit from directed treatment and achieve prolonged survival.[8,9] Panels for identification of the primary need to be flexible depending on the site of metastasis, age/gender of the patient, and detailed history which may improve sensitivity and specificity of primary detection.[10]



Figs 2A to D: (A and B) Cervical lymph node with nodal and extranodal deposits of tumor cells with papillary architecture (hematoxylin and eosin, A = ×50, B = ×100). (C and D) TTF-1 and thyroglobulin positivity is indicative of a primary in the thyroid (TTF-1 = 3,3′-Diaminobenzidine (DAB) × 100, thyroglobulin = DAB × 100)



Figs 3A to D: Biopsy from the lumbar spine with nests and clusters of neoplastic cells with positive staining for CK-20 and CDX-2 and negative staining for CK-2 (A = hematoxylin and eosin ×100, CK-20 = DAB × 100, CDX-2 = DAB × 100, CK-7 = 3,3′-Diaminobenzidine (DAB) × 100)

CONCLUSION

Immunohistochemistry can be of great help in making correct diagnosis of the primary tumor site in patients presenting with metastatic adenocarcinoma with unknown primary. To save time and resources, it is essential that sequential testing be done with basic and extended panel of IHC antibodies. Current advances in targeted therapy that has improved patient survival demand that we identify the primary site in these patients because this therapy in primary tumor is site-specific. In our study, conclusive diagnosis could be obtained in 58.82% of cases of adenocarcinoma with unknown primary by using IHC out of a total of 51 patents.

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