RESEARCH COMMUNICATION

Diagnostic Challenges and Role of Immunohistochemistry in Metastatic Liver Disease

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Abstract

Objective: To evaluate the role of Immunohistochemistry (IHC) in the diagnosis of metastatic liver disease, with a descriptive, cross-sectional study at the Department of Histopathology, Armed Forces Institute of Pathology (AFIP), Rawalpindi. Material and Methods: A total of 130 cases of metastatic liver disease were retrieved from the tumor registry data. Eosin-haematoxylin stained sections and Immunohistochemistry panels applied to ascertain the site of primary tumor were evaluated. The panels of detailed immunohistochemical markers were applied. Frequency and percentages were calculated for qualitative variables. Mean and standard deviations were calculated for quantitative variables Results: Males were 87 (67%) and were females 43 (33.07%). The most common site of primary was in GIT (45%), followed by neuroendocrine carcinoma and gall bladder. The other less common sites were lung, breast, ovary and thyroid. Conclusion: There is no specific singular panel of immunohistochemistry markers which can be used in all cases of metastatic liver tumors. The best use and selection of IHC markers depend upon morphological features, clinical history and results of other relevant investigations.

Keywords: Diagnostic approach - gastrointestinal malignancies - immunohistochemistry - metastatic liver disease

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Introduction

The liver tissue due to its peculiar blood supply is the most frequent site of metastatic disease. When compared with primary hepatic tumors; metastasis to the liver is far more common. The metastasis to liver account for about 25% of all the metastasis to solid organs. Metastatic liver disease is more common than primary liver tumors and is sometimes the initial clinical manifestation of primary in the GI tract, breast, lung, or pancreas. Adenocarcinoma is reported to be the most common tumor followed by the neuroendocrine tumors. The other tumors with frequent metastasis to liver include melanoma, lymphomas and rarely soft tissue sarcomas (Paulson et al., 2001). The radiological and ultrasound examination mostly show multiple hepatic lesions but keeping in mind the high frequency of metastatic liver disease the solitary lesions are also considered most likely to be a metastatic lesions. Pathologic evaluation of adequate biopsy samples is the main stay of making a correct diagnosis which is vital for the management of the patients. Image guided sampling using fine needle aspiration (FNA) or needle core biopsy (NCB) of the mass lesions or computed tomography (CT) guided biopsy is usually performed to provide tissue for histopathological evaluation. The routine biopsy techniques usually provides diagnostic sample in more than 90% of the cases. The choice of FNA, NCB or endoscopic ultra sound guided FNA (EUS-FNA) depends upon the size and site of the lesion, experience of radiologist or clinician performing the biopsy. FNA with good cell block in usually sufficient to reach the final diagnosis. There is no doubt that adequate sampling from representative area is vital for the final diagnosis (Axe et al.,1986; Centeno, 2000; Steward et al., 2000).

Pathologists are always confronted with issues like identifying the tumor type, differentiating between the primary hepatic neoplasm and secondary tumor. Only on routinely stained sections it is difficult, sometimes impossible to differentiate between cholangiocarcinoma and metastatic adenocarcinoma. It is important to establish the tumor type as first step i.e. to differentiate lesion as carcinoma, sarcoma, lymphoma, neuroendocrine or melanoma. Distinguishing hepatocellular carcinoma from metastatic lesion with similar morphology is difficult. Renal cell carcinoma, adrenal cortical carcinoma and even adenocarcinoma pose difficult problem on evaluation of H&E stained sections. In some of the cases accurate identification of the tumors is possible but sub classification of carcinomas of unknown primary always remains problematic. Availability of adequate clinical history is of great importance to the pathologist. Radiological findings, serum tumor markers

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and any past history of neoplastic lesion is pertinent.

Combination of morphologic evaluation of haematoxylin and eosin (H&E) stained sections and immunohistochemistry is the standard for pathologic practice. Multidisciplinary approach utilizing all available clinical information and selection of appropriate immunohistochemical makers usually help in cases with unknown primary (Steward et al., 2002). The rationale of the study is to evaluate the role of immunohistochemistry in metastatic liver disease.

Materials and Methods

A descriptive cross sectional study was carried out in the department of histopathology, Armed forces institute of pathology (AFIP) during the period from Jan 2008 to 30 Sep 2010. The AFIP receives specimens from all over Pakistan from civil and army hospitals. Cases of metastatic liver disease irrespective of age and gender, in which immunohistochemistry was applied to find out the primary were retrieved from the A.F.I.P tumor registry record. The slides were reviewed for analysis of various immunohistochemical markers. Clinical history including radiological finding, relevant serum tumor markers and data regarding the gender and age of the patients was extracted from the laboratory request forms including any additional information as received from clinicians.

A total of 130 cases of metastatic liver tumors were diagnosed during this period. Needle core biopsy (NCB) was received in 85%, fine needle aspiration biopsy (FNA) and cell block were in 10%. Rest were open biopsy specimen along with cholecystectomy specimen. The IHC markers included CK AE1/AE3,CEA,CA-125,CK7, CK20, TTF1, Hep-Par1, AFP, RCC, PSA, PSAP, Chromagranin , Neuron specific enolase (NSE), Vimentin, Desmin, LCA, GCDFP-15, CD 56, CD30, Melan A, Inhibin, and Calretenin. Data was analyzed on SPSS (Statistical package for social sciences) version 17. Mean, median and mode was calculated for quantitative variable like patient's age. Frequencies and percentages were calculated for qualitative variables like histological tumour type, and results of immuno-histochemical markers.

On the average four antibodies were applied before predicting the primary site of tumor. In cases of suspected adenocarcinoma IHC markers included CK AE1/AE3, CEA, CA-125, CK7, CK20, CK19 and TTF1. To differentiate adenocarcinoma from primary hepatocellular carcinoma and Hep-Par1, AFP, CK7, CK20 and CEA was applied. The RCC anti body was applied in suspected primary in kidney. In male patients with unknown primary with no clinical details, PSA, PSAP and PLAP was applied to reach the final diagnosis. Chromagranin and Neuron specific enolase (NSE) was applied in cases with features of neuroendocrine differentiation on H & E stained sections. Other markers applied included Vimentin, Desmin, LCA, GCDFP-15, CD 56, CD30, CD20, Inhibin, and Calretenin.

Table 1. Immunohistochemical Markers for the Differential Diagnosis of Hepatocellular carcinoma, and Metastatic Adenocarcinoma

IHC marker	Hepatocellular carcinoma	Adenocarcinoma
Hep Par-1	+	-
CEA	Canalicular	+
AFP	+	-
CK7	+	+
CK20	-/Rarely positive	+

Table 2. Cytokeratin Expression in Various Types of Adenocarcinoma

CK7+/CK20+	CK7+/CK20-	CK7-/CK20+	CK7-/CK20-
Urothelial carc	Breast	Colorectal	Prostate
Pancreas	Lungs		RCC
Biliary tract	Esophagus/stomach		HCC
Cholangiocarc	Pancreas		ACC
Eso/stomach	Biliary tract		
Mucinous carc	Cholangiocarcinoma		
	Ovary, Endometrium		

RCC, Renal cell carcinoma; HCC, Hepatocellular carcinoma; ACC, adrenal cortical carcinoma; *ovarian, colon, mucinous brochoalveolar

Results

A total of 130 cases of metastatic liver disease were included. Eighty seven (67%) were males and forty three (33 %) were females. Mean age was 51 + 13.7 years with age range of 6 to 80 years. The most common site of primary was gastrointestinal tract (G.I.T) comprising 59 cases (45.3 %) followed by neuroendocrine tumors 14 cases (10.7 %), gall bladder in 13 cases (10 %), lung 8 cases (6.15 %), pancreas 6 cases (4.6 %), ovary 5 cases (3.86 %), renal 4 cases (3 %), Hodgkin's lymphoma, thyroid 3 cases each (2.30 %), leiomyosarcoma , squamous cell carcinoma, breast, carcinoid 2 cases each (1.5 %) and Non-Hodgkin's lymphoma, seminoma, prostatic carcinoma ,uterus 1 cases each (0.76 %). Most of the tumors presented in sixth decade (30%), followed by seventh (20.7%), fifth (20%), fourth decade (15.38%) and eight decade (10%). There were only three (2.3%) cases in third decade and (1.6%) cases in second decade.

Table 1 shows IHC markers used for the differential diagnosis of primary hepatic carcinoma and metastatic carcinoma. Table 2 shows coexpression of CK7/CK20 in various adenocarcinomas. Comparison of the various combinations allowed distinction in many of the sites of primaries as compared to HCC. Detailed clinical history along with ultrasound examination, CT imaging results and biochemical results of tumor markers were available before the application of immunohistochemistry markers in 45% of the cases.

In rest of the cases subsequent investigations were carried out in the light of cytoarchitectural features as observed on H&E stained sections and results of initial panel of IHC markers. Multiple lesions were seen in 55% of the cases and 45% presented as single hepatic mass lesion. In cases of poorly differentiated tumors Melan A

was also applied but no case of metastatic melanoma was diagnosed in our study.

Discussion

Liver is the second most common site of metastatic lesions after lymph node. It is frequently biopsied for ascertaining the site of primary. Most of the liver metastasis are multiple and same was our observation. In majority cases (77%) both lobes are involved. The identification of primary is important for the management of metastatic liver disease (Lygidakis and Pear, 1997). The basic and crucial step is to establish a tumor type on the basis of histomorphological features. The diagnostic dilemma arises in cases of poorly differentiated tumors and without clinical details. To make appropriate selection of immunohistochemistry markers all the above information requires detailed evaluation. In most of the cases morphology is helpful in differentiation between carcinoma, sarcoma, lymphoma or melanoma. Panel including cytokeratin, S100 and Leukocyte common antigen (LCA) can assist to confirm the primary impression. Additional antibodies are added once the diagnosis has been narrowed (Ohlsson B et al., 1993; Abbruzzese JL et al., 1995).

International studies reported bimodal age distribution as in this study. Carcinoma of Lung, colon, pancreas, breast and stomach are the most primary sites metastasizing to liver. The most common site of primary in our study was from G.I.T followed by neuroendocrine as was reported earlier (Ohlsson B et al., 1993).

Another important point is to differentiate metastatic tumors from primary hepatic malignancies. Hepatocellular carcinomas can mimic metastatic adenocarcinomas, renal cell carcinomas, adrenocortical tumors and cholangiocarcinomas (Lewis RB et al., 2010; Mayo and Pawlik, 2009). A panel of immunohistochemical markers comprising CK7, CK20, CEA, CD10, AFP and Hep-Par1 are useful in differentiating metastatic from primary lesions (Abbruzzese JL et al., 1995). The CEA gives Canalicular staining pattern in hepatocellular carcinomas while in metastatic adenocarcinomas its gives cytoplasmic staining of the tumor cells.

The helpful morphological features of adenocarcinomas include columnar cells forming glandular structures and may show mucin production, within either the cytoplasm or lumen of the glandular structures. Stains such as mucicarmine can help to demonstrate it. Morphological subtypes like signet ring cell carcinoma, mucinous carcinoma or colloid type carcinoma can be identified on detailed examination of multiple sections. These histological feature help to narrow down the possible differential diagnosis for the primary tumor. It is commonly observed that morphology of adenocarcinoma from lungs, endometrium, esophagus or intestinal types of gastric carcinoma do not have any specific features. Mucinous carcinomas constitute subtype of adenocarcinomas characterized by cytoplasmic or extracellular mucin production. In addition to tumors from colon mucinous variety also occurs in breast, ovaries, and pancreas and may arise anywhere in GI tract. Metastatic lesions from mucinous bronchioalveolar carcinoma show similar feature. The metastatic deposits in liver sometimes show malignant glandular structures floating in mucin or pool of mucin with scattered malignant cells. In case of signet cell carcinomas the nucleus is pushed to one side giving it appearance of signet cell. Gastric carcinoma may show this typical appearance. The useful Immunohistochemical panel in such cases includes CK7, CK20, estrogen receptor and GCDFP15. Additional marker such as calretenin are required to identify nonmucinous adenocarcinoma from lung (Mayo and Pawlik, 2009; Geller SA et al.,2008; Pavlidis and Fizazi, 2005; Varadhachary GR et al., 2004)

The second most common tumors metastasizing to liver are neuroendocrine carcinomas. Neuroendocrine carcinomas show varying degree of differentiation. Monomorphic pattern is common in low grade tumors as carcinoids with minimal mitotic activity and without any necrosis. Tumors with plasmacytoid appearance on cytological smears are pathognomonic. The classical salt and pepper appearance of cytoplasm both on cytological smears and core biopsy also help to diagnose neuroendocrine tumors. High grade tumors such as small cell carcinoma from lung usually show nuclear molding, necrosis and high mitotic rate. The chromatin in these cases is finely stippled the standard panel in these cases include chromagranin, synaptophysin and CD56 (Tot, 2000; Lisa M et al.,1991).

Lymphomas characteristically show dyshesive. In our study one case of Non-Hodgkin's lymphoma (Diffuse large B cell type) was diagnosed by using LCA, CD20, and CD3 along with cytokeratin panel.

In small percentage of the cases immunohistochemical marker may not resolve the dilemma requiring careful interpretation and revisiting of basic histomorphological features as main diagnostic criteria.

The most common site of metastatic liver disease in our study was G.I.T followed by neuroendocrine carcinomas. Males are more commonly affected with the majority of the patients presenting in the sixth decade of life. There is no single panel of Immunohistochemical markers that can be applied. The scantiness of available material requires careful evaluation of clinical and radiological correlation before selecting immunohistochemical panel. In some cases final diagnosis may require specialized investigations before reaching to a final diagnosis.

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