

Immunohistochemical Distinction of Hepatocellular Carcinoma Using Arginase-1, Hepatocyte Paraffin Antigen -1 and Glypican-3

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ABSTRACT

AIM: The distinction of hepatocellular carcinoma (HCC) from the metastatic carcinoma (MC) and intrahepatic cholangiocarcinoma (CC) often presents a diagnostic challenge that carries a significant impact on its subsequent management. In this study we aimed to evaluate the immunohistochemical expressions of arginase-1, hepatocyte paraffin antigen-1 (HepPar-1), and glypican-3 (GPC-3) in a trial to distinguish HCC from non-HCC involving the liver.

MATERIALS AND METHODS: The study included 64 cases (32 HCC, 28 MC and 4 CC) and 5 specimens of normal liver tissues. These cases were investigated retrospectively from the archive of the Pathology Department, Zagazig University Hospitals. The predictive capacity of arginase-1, HepPar-1 and GPC-3 staining was determined using sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) calculations.

RESULTS: Both arginase-1 and HepPar-1 expressions were present in all cases of non-neoplastic hepatocellular tissues, whereas GPC-3 expression was absent in all cases. Only two of 28 (7.1%) cases of MC and one of 4 (25%) cases of CC showed positive immunoreactivity for arginase-1. HepPar-1 immunoreactivity was detected in 3 of 28 (10.7%) cases of MC and in one of 4 (25%) cases of CC. GPC-3 immunoreactivity was detected in 2 of 28 (7.1%) cases

of MC and negative in all cases of CC. Arginase-1 was more sensitive (87.5%) than HepPar-1 (71.9%) or GPC-3 (65.6%) for HCC. GPC-3 was more specific (93.8%) than HepPar-1 (87.5%) and arginase-1 (90.6%). However, the combination of the three biomarkers for the diagnosis of HCC raised the specificity to 100%.

CONCLUSION: Arginase-1 and HepPar-1 are effective biomarkers for HCC differentiation. Also, arginase-1 demonstrates a superior sensitivity in comparison with GPC-3 and HepPar-1 in the diagnosis of HCC, whereas GPC-3 demonstrates superior specificity. Hence, the use of combination of arginase-1 with HepPar-1 and GPC-3 could be useful in the precise diagnosis of HCC and distinguishing it from non-HCC.

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Key words: Arginase-1; Hepatocyte Paraffin Antigen-1; Glypican-3; Hepatocellular Carcinoma; Immunohistochemistry

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INTRODUCTION

Hepatocellular Carcinoma (HCC) is the most common primary cancer of liver, representing the third most common cause of cancer deaths all over the world^[1]. Egypt has a high prevalence of HCC; it is the second most cancer site among males after cancer bladder and seventh among females^[2]. Such high incidence has been attributed to the high prevalence of hepatitis C virus (HCV) amongst Egyptians^[3].

Based on histological characters, most HCCs can be easily identified on hematoxylin and eosin stained sections. However, some types of benign and malignant tumors may share the morphologic features. HCC mimickers include some cases of adrenocortical carcinoma, renal cell carcinoma (RCC), large cell neuroendocrine carcinoma, clear cell sarcoma, melanoma and angiomylipoma^[4,5]. In

addition, the poorly differentiated HCC may be difficult to identify based on morphology alone^[6].

The distinctions of HCCs from metastatic tumors in the liver usually present a diagnostic challenge, especially in cases of small tissue biopsies or fine-needle aspiration (FNA) biopsy specimens that carries significant impact on subsequent management^[4].

There is a limited number of ancillary immunohistochemical biomarkers used for distinguishing HCC from adenocarcinoma including HepPar-1, polyclonal carcinoembryonic antigen (CEA), and cluster of differentiation 10 (CD10), with Alfa-fetoprotein (AFP) and GPC-3^[3]. However, the suboptimal sensitivity and difficulty in interpretation of each one of such biomarkers renders its utility to be limited^[7].

HepPar-1 is a mitochondrial urea cycle antigen. It has been increasingly used as a positive biomarker for hepatic differentiation, but does not distinguish benign from malignant hepatocytes^[8]. In addition, this biomarker has a relatively low sensitivity in poorly differentiated HCC so its distinction from adenocarcinoma is difficult^[7,9].

GPC-3 is a member of the glypican family of heparan-sulfate proteoglycans. It is bound to the plasma membrane through a glycosyl phosphatidyl-inositol (GPI) anchor. It is specifically appeared in fetal hepatoblasts and is silenced in normal tissues of adult liver^[9]. Its expression tends to reappear with malignant transformation^[10]. It shows a high specificity with suboptimal sensitivity in the diagnosis of HCC. Also, it shows immunoreactivity in many other tumors, such as pulmonary squamous cell carcinoma^[12], germ cell tumors^[13] and subtypes of gastric adenocarcinomas^[14].

A new immunohistochemical marker, arginase-1, has been identified with possible utility in differentiating HCC from metastatic adenocarcinoma^[4]. It is found in 2 isoforms, namely arginase-1 and arginase-2, both of which are metalloenzymes responsible for the hydrolysis of arginine to ornithine and urea in the urea cycle. Arginase-1 is expressed in normal human liver with a high degree of specificity^[15], whereas arginase-2 level is highest in the kidneys and pancreas and is very low in the liver^[16].

The current study aimed to demonstrate the expressions of arginase-1, HepPar-1 and GPC-3 in cases of HCC, CC and MC involving the liver. This is a trial to evaluate their diagnostic utility in distinguishing HCC from non-hepatocellular carcinomas.

MATERIALS AND METHODS

Tissue specimens

The study included 32 cases of HCC (26 primary HCC and 6 metastatic HCC), 28 cases of MC to the liver from varying sites, 4 cases of intrahepatic CC and 5 specimens of normal liver tissues served as control cases. These cases were studied retrospectively from the archive of the Pathology Department, Zagazig University Hospitals during the period between 2004 and 2015. The clinicopathological data, histological sections and relevant ancillary diagnostic stains were reviewed to confirm diagnosis. This study matched with the local ethics approval.

The histological grading of HCCs was established using WHO criteria^[17]. For grade I (well differentiated) HCC, the nuclear features were similar to those of hepatocellular adenoma; and the diagnosis of HCC was based on focal atypical features such as small cell change and widened cell plates. In grade II (moderately differentiated) HCC, the tumor grade was increased with increased nuclear/cytoplasmic ratio, prominent nucleoli, nuclear membrane irregularities and nuclear pleomorphism. The diagnosis of grade 3 (poorly differentiated) HCC

was based on the combination of clinical parameters (elevated serum AFP level and absence of another primary source of tumor) and histological features (bile production by tumor cells, adjacent area of better differentiated HCC and immunophenotype).

Immunohistochemical procedure

Immunohistochemical staining was performed using 4- μ m thick sections. The sections were put in the oven for 30 minutes and then de-paraffinized with 2 changes of xylene at 3 minutes each, and subsequently rehydrated in 4 changes of decreasing alcohol concentrations at 3 minutes for each change. After rinsing in phosphate buffered saline (PBS), antigen retrieval was performed by treating the tissue sections with 0.1 mol/L citrate buffer, pH 6.0 for 10 min in a microwave at 100°C for 20 minutes, then left to cool at room temperature for 20 minutes. The endogenous peroxidase activity was blocked by incubating the slides in 3% hydrogen peroxide for 5 to 10 min, and then washed in buffer. At room temperature, the sections were placed overnight with a rabbit polyclonal antibody against arginase-1 (H-52: sc 20150, Santa Cruz, Europe at a dilution 1:200), a mouse monoclonal antibody against HepPar-1, (clone OCH1E5, MS-1810-R7, 1:100 dilution, Lab vision, CA, USA) and monoclonal antibody against glypican-3 (1: 100, clone 1G12, Biocare Medical, USA). After rinsing in PBS, the tissues were incubated a biotin-free horseradish peroxidase-labeled dextrose-based polymer complex bound to secondary antibody (DAKO EnVision Plus System-HRP (DAB), DakoCytomation, Carpinteria, CA). The peroxidase reaction was visualized by incubating the sections with diaminobenzidine (DAB). The sections were counterstained with Mayer's hematoxylin followed by dehydration, clearing and mounting.

The entire procedures were performed at room temperature. Normal liver tissues were used as positive controls, while negative controls had primary antibody replaced by buffer and were run with the patient slides.

Immunohistochemical evaluation

Only cytoplasmic or cytoplasmic and nuclear reactivity was considered as a positive staining for arginase-1. As regard HepPar-1, positivity was defined as coarsely granular cytoplasmic staining that could not be confused with background staining. Cytoplasmic staining of glypican-3 was considered as positive. Immunoreactivity was semi-quantitatively and independently scored by 2 surgical pathologists (AE and HR). The intensity of immunostaining was scored as 0 (no staining), 1+ (weak staining), and 2+ (strong staining). Furthermore, the pattern of staining was recorded and scored as negative if <5% of tumor or lesional cells were stained, focal staining was defined as reactivity in 5%-50% of tumor or lesional cells and diffuse if >50% of tumor or lesional cells were stained^[18].

Statistical analysis

Categorical variables were expressed as a number (percentage). Validity of Arginase-1, HepPar-1, and GPC-3 and their combinations in distinguishing HCC from non-hepatocellular tumors (CC and MC) was calculated using diagnostic performance depending on sample 2x2 contingency tables. The sensitivities, specificities, positive predictive values (PPV), negative predictive values (NPV), and accuracies with their respective 95% confidence intervals were calculated. The histological diagnosis designated as the gold standard. All tests were two sided. A P-value <0.05 was considered significant. All statistics were performed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) & MedCalc 13 for windows (MedCalc Software bvba).

Table 1 Association of Arginase-1, HepPar-1, and GPC-3 extension with histopathological diagnosis.

Histopathological diagnosis	No	Arginase-1 extension			HepPar-1 extension			GPC-3 extension		
		Total +ve	Diffuse	Focal	Total +ve	Diffuse	Focal	Total +ve	Diffuse	Focal
HCC	32	28 (87.5%)	23 (82.1%)	5 (17.9%)	23 (71.9%)	15 (65.2%)	8 (34.8%)	21 (65.6%)	14 (66.7%)	7 (33.3%)
Well differentiated	8	8 (100%)	8 (100%)	0 (0%)	8 (100%)	7 (87.5%)	1 (12.5%)	4 (50%)	3 (75%)	1 (25%)
Moderately differentiated	14	12 (85.7%)	10 (83.3%)	2 (16.7%)	11 (78.5%)	7 (63.3%)	4 (36.7%)	10 (71.4%)	7 (70%)	3 (30%)
Poorly differentiated	10	8 (80%)	5 (62.5%)	3 (37.5%)	4 (40%)	1 (25%)	3 (75%)	7 (70%)	4 (57.1%)	3 (42.9%)
Metastatic carcinoma	28	2 (7.1%)	2 (100%)	0 (0%)	3 (10.7%)	1 (33.3%)	2 (66.7%)	2 (7.1%)	1 (50%)	1 (50%)
Colon	10	0 (0%)	0 (0%)	0 (0%)	2 (20%)	0 (0%)	2 (100%)	1 (10%)	1 (100%)	0 (0%)
Pancreas	4	1 (25%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Gastric	2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Breast	2	1 (50%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	0 (0%)	1 (100%)
Prostate	2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Pulmonary	4	0 (0%)	0 (0%)	0 (0%)	1 (25%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Neuroendocrine	1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Conventional RCC	3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Cholangiocarcinoma	4	1 (25%)	1 (100%)	0 (0%)	1 (25%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Cirrhotic liver tissues adjacent to HCC	18	18 (100%)	18 (100%)	0 (0%)	18 (100%)	18 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Non-neoplastic liver tissue adjacent to metastatic carcinoma	10	10 (100%)	10 (100%)	0 (0%)	10 (100%)	10 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Normal liver tissues	5	5 (100%)	5 (100%)	0 (0%)	5 (100%)	5 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Qualitative data are presented as number (%).

RESULTS

Clinicopathological findings

The age of patients ($n = 64$) at the time of initial diagnosis ranged from 40 to 70 years; the mean age was 45.7 ± 15.7 years. The patients were 44 males and 20 females. Ultrasonographic data obtained from the patients' reports revealed the presence of cirrhosis in 90 % of HCC patients. In contrast, cirrhosis was not evident in all cases of non-hepatocellular carcinoma. All selected cases were from tumors >1 cm in size.

The 32 cases of HCC were graded as 8 well differentiated, 14 moderately differentiated, and 10 poorly differentiated. Eighteen cases were surgically resected specimens and had adjacent non-neoplastic liver tissues that revealed cirrhosis and 14 were needle core biopsies. Only six cases of HCC were biopsies of metastatic sites (bone "3", portahepatis, retroperitoneum and adrenal gland) and the remaining 26 were primary in the liver. The 28 needle core biopsies of MC to the liver included in this study had well-documented known primary sites, including the colorectum (10 cases), pancreas (4 cases), breast (2 cases), lung (4 cases), prostate (2 cases), stomach (2 cases), renal cell carcinoma (3 cases) and one case of neuroendocrine carcinoma. Four adenocarcinoma involved the liver had no other anatomic site of disease; and were classified as intrahepatic CC and confirmed by relevant ancillary diagnostic stains. The non-neoplastic liver tissues adjacent to MC were detected in 10 cases.

Immunohistochemical results

Immunohistochemical expressions of arginase-1, HepPar-1 and GPC-3 in all the studied cases are summarized in Tables 1 and 2. All normal liver tissues ($n=5$), non-neoplastic cirrhotic liver tissues adjacent to HCC ($n=18$) as well as liver tissue adjacent to MC ($n=10$) showed diffuse and strong (2+) immunostaining for both arginase-1 and HepPar-1. In contrast, GPC-3 was negative in all these cases.

In HCC, arginase-1 showed immunoreactivity in 28 of 32 (87.5%) cases (Figs. 1-3). Of 28 positive arginase-1 HCC cases, 23 (82.1%) exhibited diffuse staining involving $>50\%$ of tumor cells, whereas only 5 cases (17.9%) demonstrated focal staining. As regard to staining intensity, 17/28 (60.7%) exhibited a strong staining but 11/28 (39.3%) demonstrated a weak staining. HepPar-1 demonstrated

immunoreactivity in 23 of 32 (71.9%) hepatocellular carcinomas. The staining was diffuse in 15/23 (65.2%) and focal in 8/23 (34.8%). Of 23 positive HepPar-1 HCC cases, 14 (60.9%) exhibited a strong staining, whereas 9 cases (39.1%) demonstrated a weak staining. Of the 10 poorly differentiated HCC cases, 8 (80%) were positive for arginase-1 (62.5% diffuse and 75% were weak) but only four cases (40%) demonstrated HepPar-1 positivity (75% were focal and 100% were weak). In all studied HCC cases, there were no cases positive for HepPar-1 with concurrent negative arginase-1 staining, while 5 HCC cases showed arginase-1 positive staining but were negative for HepPar-1.

GPC-3 demonstrated immunoreactivity in 21 of 32 (65.6%) cases of HCC. The staining was diffuse in 14/21 (66.7%) and focal in 7/21 (33.3%). Of the 21 positive GPC-3 HCC cases, 11 (52.4%) exhibited a strong staining, whereas 10 cases (47.6%) demonstrated a weak staining. In poorly differentiated HCC, GPC-3 demonstrated immunoreactivity in 70% of cases (57.1% of positive cases were diffuse and 71.4% were weak).

Among all HCC cases, arginase-1 showed a significantly higher sensitivity for diagnosis of HCC (87.5%) compared to HepPar-1 (71.9%) ($p=0.001$) and GPC-3 (65.6%) ($p=0.003$). In contrast, non-significant difference between HepPar-1 and GPC-3 was found ($p=0.423$). There was statistically significant difference in HepPar-1 expression among the different grades of HCC ($p=0.015$). However, there was no statistically significant difference in GPC-3 ($p=0.560$) or arginase-1 ($p=0.428$) expression among the different grades of HCCs (Table 3).

Within the different grades of HCC; the sensitivities of arginase-1 in well, moderately, and poorly differentiated HCCs were 100%, 85.7%, and 80%, respectively, whereas, in comparison, HepPar-1 demonstrated sensitivities of 100%, 78.6%, and 40% for well, moderately, and poorly differentiated tumors, respectively. In addition, GPC-3 demonstrated sensitivities of 50%, 71.4%, and 70% for well, moderately, and poorly differentiated tumors, respectively. There was no significant difference between arginase-1 and HepPar-1 as regards their sensitivities in diagnosis of well or moderately differentiated HCC, while for poorly differentiated HCC cases; arginase-1 showed a significantly higher sensitivity than HepPar-1 ($p=0.001$). In well differentiated HCC cases, arginase-1 showed a significantly higher sensitivity than GPC-3 ($p=0.001$).

Table 2 Association of Arginase-1, HepPar-1, and GPC-3 intensity with histopathological diagnosis.

Histopathological diagnosis	No	Arginase-1 intensity			HepPar-1 intensity			GPC-3 intensity		
		Total +ve	Strong	Weak	Total +ve	Strong	Weak	Total +ve	Strong	Weak
HCC	32	28 (87.5%)	17 (60.7%)	11 (39.3%)	23 (71.9%)	14 (60.9%)	9 (39.1%)	21 (65.6%)	11 (52.4%)	10 (47.6%)
Well differentiated	8	8 (100%)	7 (87.5%)	1 (12.5%)	8 (100%)	8 (100%)	0 (0%)	4 (50%)	3 (75%)	1 (25%)
Moderately differentiated	14	12 (85.7%)	8 (66.7%)	4 (33.3%)	11 (78.6%)	6 (54.5%)	5 (45.5%)	10 (71.4%)	6 (60%)	4 (40%)
Poorly differentiated	10	8 (80%)	2 (25%)	6 (75%)	4 (40%)	0 (0%)	4 (100%)	7 (70%)	2 (28.6%)	5 (71.4%)
Metastatic carcinoma	28	2 (7.1%)	2 (100%)	0 (0%)	3 (10.7%)	1 (33.3%)	2 (66.7%)	2 (7.1%)	2 (100%)	0 (0%)
Colon	10	0 (0%)	0 (0%)	0 (0%)	2 (20%)	1 (50%)	1 (50%)	1 (10%)	1 (100%)	0 (0%)
Pancreas	4	1 (25%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Gastric	2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Breast	2	1 (50%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	1 (100%)	0 (0%)
Prostate	2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Pulmonary	4	0 (0%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)
Neuroendocrine	1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Conventional RCC	3	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Cholangiocarcinoma	4	1 (25%)	1 (100%)	0 (0%)	1 (25%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)
Cirrhotic liver tissues adjacent to HCC	18	18 (100%)	18 (100%)	0 (0%)	18 (100%)	18 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Non-neoplastic liver tissue adjacent to metastatic carcinoma	10	10 (100%)	10 (100%)	0 (0%)	10 (100%)	10 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Normal liver tissues	5	5 (100%)	5 (100%)	0 (0%)	5 (100%)	5 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Qualitative data are presented as number (%).

Table 3 Association of Arginase-1, HepPar-1, and GPC-3 intensity with histopathological diagnosis.

Histopathological diagnosis	No	Arginase-1 staining			HepPar-1 staining			GPC-3 staining		
		-ve	+ve	p*	-ve	+ve	p*	-ve	+ve	p*
HCC		(n=4)	(n=28)		(n=9)	(n=23)		(n=11)	(n=21)	
Grade I	8	0 (0%)	8 (100%)		0 (0%)	8 (100%)		4 (50%)	4 (50%)	
Grade II	14	2 (14.3%)	12 (85.7%)	0.428	3 (21.4%)	11 (78.6%)	0.015	4 (28.6%)	10 (71.4%)	0.560
Grade III	10	2 (20%)	8 (80%)		6 (60%)	4 (40%)		3 (30%)	7 (70%)	

Qualitative data are presented as number(%),* Chi-square test, p <0.05 is significant.

Only 2 of 28 (7.1%) cases of MC (1 pancreatic and 1 breast) and one of 4 (25%) cases of CC showed positive immunoreactivity for arginase-1 and the staining was strong and diffuse (Figures. 4,5). HepPar-1 immunoreactivity was detected in 3 of 28 (10.7%) cases of MC (2 colonic and 1 pulmonary) and in 1/4 (25%) cases of CC. In contrast, GPC-3 was positive in 2 of 28 (7.1%) metastatic adenocarcinomas which were 1 colonic adenocarcinoma and 1 breast carcinoma. In addition, GPC-3 was negative in all cases of CC (Figure 4).

Statistical analysis for individual markers and select marker combinations is presented in table 4. Overall, arginase-1 is a more sensitive (87.5%) marker of hepatic differentiation than HepPar-1 (71.9%) or GPC-3 (65.6%). However, GPC-3 is somewhat more specific (93.8%) than arginase-1 (90.6%) or HepPar-1 (87.5%).

The PPV for arginase-1 in distinguishing HCC from other non-HCC tumors was better (90.3%) than observed with HepPar-1 (85.2%); however, both biomarkers were inferior to GPC-3 in this regard, which demonstrated a PPV of 91.3%. The NPV for arginase-1 (87.9%) in distinguishing HCC from other non-HCC tumors was better than that of both HepPar-1 (75.7%) and GPC-3 (73.2%), respectively.

The combination of either arginase-1 or HepPar-1 staining for a diagnosis of HCC showed a sensitivity of 87.5% and specificity of 78.1%. Requiring both arginase-1 and HepPar-1 immunoreactivity for a diagnosis of HCC yielded a specificity of 100% with a diminished sensitivity to 71.9%. The combination of either arginase-1 or GPC-3 staining for a diagnosis of HCC revealed a sensitivity of 87.5% and specificity of 87.5%. Requiring both arginase-1 and GPC-3 immunoreactivity for a diagnosis of hepatocellular carcinoma yielded a specificity of 96.9% with a diminished sensitivity to 65.6%.

DISCUSSION

The differential diagnosis of HCCs from metastatic tumors and intrahepatic CC is extremely useful for subsequent prognostication and management. We evaluated the immunohistochemical expression of arginase-1 in cases of HCC, MC and intrahepatic CC as compared to HepPar-1 and GPC-3. There was a diffuse and strong immunostaining for both arginase-1 and HepPar-1 in normal liver tissue and the cirrhotic liver tissues adjacent to HCC as well as liver tissue adjacent to MC whereas, negative GPC-3 staining was observed in all these cases. Unlike GPC-3, arginase-1 has no role in distinguishing well-differentiated hepatocellular carcinoma from benign hepatic lesions, as arginase-1 demonstrated a diffuse strong reactivity in non-neoplastic liver. This confirms the study of Timek et al^[18] and Fujiwara et al^[19], who reported that arginase-1 has no role in distinguishing well-differentiated hepatocellular carcinoma from benign hepatic lesions.

The current results revealed that arginase-1 showed a significantly higher overall sensitivity for diagnosis of HCC (87.5%) compared to HepPar-1(71.9%) and GPC-3 (65.6%). This confirms the conclusion of the previous studies^[18-20]. McKnight et al^[20], performed arginase-1 IHC on FNA cell block (CB); and its performance characteristics were compared with HepPar-1 and GPC-3. They reported that arginase-1 positivity was demonstrated in 84.1% of HCC, compared to 72.7% and 56.8% of HepPar-1 and GPC-3, respectively. In addition, Fujiwara et al^[19], evaluated 98 fine needle aspiration biopsies (FNABs) and found the sensitivity of arginase-1 as 81%, HepPar-1 as 70% and GPC-3 as 54% for HCC.

It is worth mentioning that in the current study, there were no cases positive for HepPar-1 with concurrent negative arginase-1 staining suggesting that Arg-1 may substitute for HepPar-1 in diagnostic immunohistochemistry. In addition, arginase-1 showed more diffuse

Table 4 Diagnostic performance of Arginase-1, HepPar-1 and GPC-3 in distinguishing Hepatocellular Carcinoma (HCC) from non-hepatocellular tumors (cholangiocarcinoma and metastatic carcinoma).

Marker and Marker Combinations	True +ve	False +ve	True -ve	False -ve	SN % (95% CI)	SP % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Acc (95% CI)
Arginase-1	28	3	29	4	87.5% (76-99)	90.6% (80.5-100)	90.3% (79.9-100)	87.9% (76.7-99)	89.1% (81.4-96.7)
HepPar-1	23	4	28	9	71.9% (56.3-87.5)	87.5% (76-99)	85.2% (71.8-98.6)	75.7% (61.9-89.5)	79.7% (69.8-98.5)
GPC-3	21	2	30	11	65.6% (49.2-82.1)	93.8% (85.4-100)	91.3% (79.8-100)	73.2% (59.6-86.7)	79.7% (69.8-89.5)
Arginase-1 and Glypican-3	21	1	31	11	65.6% (49.2-82.1)	96.9% (90.8-82.1)	95.5% (86.8-100)	73.8% (60.5-87.1)	81.3% (71.7-90.8)
Arginase-1 or Glypican-3	28	4	28	4	87.5% (76-99)	87.5% (76-99)	87.5% (76-99)	87.5% (76-99)	87.5% (79.4-95.6)
Arginase-1 and HepPar-1	23	0	32	9	71.9% (56.3-87.5)	100%	100%	78% (65.4-90.7)	85.9% (77.4-94.5)
Arginase-1 or HepPar-1	28	7	25	4	87.5% (76-99)	78.1% (63.8-92.4)	80% (73.7-98.8)	86.2% (73.7-98.8)	82.8% (73.6-92.1)
Arginase-1, HepPar-1 and Glypican-3	16	0	32	16	50% (32.7-67.3)	100%	100%	66.7% (53.3-80)	75% (64.4-85.6)
Arginase-1 or HepPar-1 or Glypican-3	28	7	25	4	87.5% (76-99)	78.1% (63.8-92.4)	80% (73.7-98.8)	86.2% (73.7-98.8)	82.8% (73.6-92.1)

SN: Sensitivity, SP: Specificity, PPV: Positive Predictive Value, NPV: Negative Predictive Value, Acc: Accuracy, 95%CI: 95% Confidence Interval.

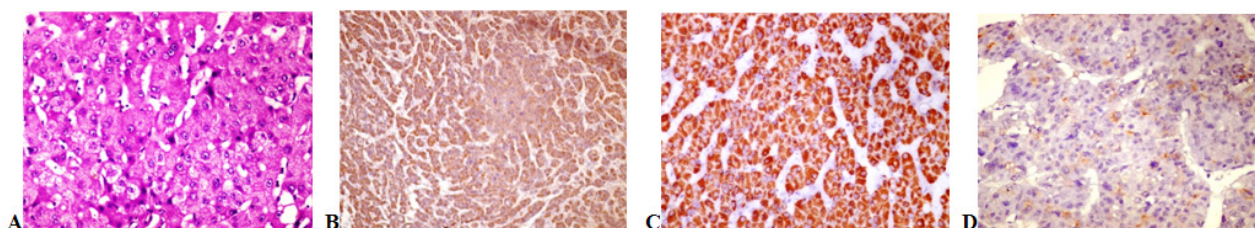


Figure 1 A: a case of well differentiated hepatocellular carcinoma (H&E x400); B: shows strong and diffuse arginase-1 staining (x100); C: Shows strong and diffuse HepPar-1 Immunostaining (x400); D: Shows focal Glypican-3 immunostaining (x400).

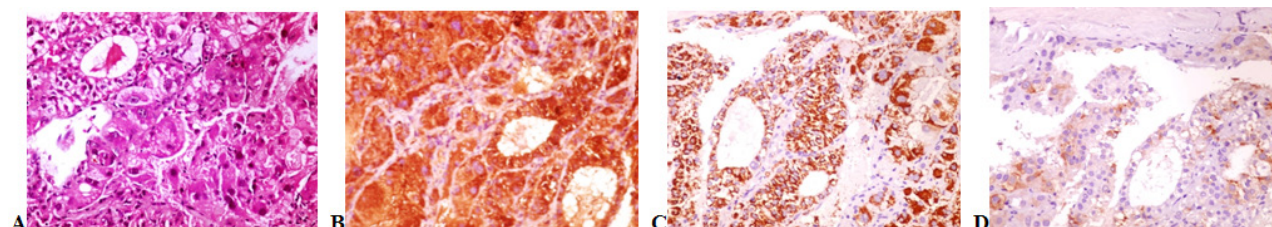


Figure 2 A: a case of moderately differentiated hepatocellular carcinoma with pseudo-glandular structures (H&E x400); B: shows strong and diffuse arginase-1 staining (x400); C: shows strong and diffuse HepPar-1 immunostaining (x400); D: shows focal Glypican-3 immunostaining (x400).

staining in HCC (82.1%) than HepPar-1 (65.2%) and GPC-3 (66.7%). This makes interpretation of arginase-1 is easier especially in small or fragmented liver biopsies.

The most difficult histological differentiation is between poorly differentiated HCC and adenocarcinoma^[7,9]. In this setting, arginase-1 exhibited higher sensitivity than HepPar-1, which demonstrated low immunoreactivity in poorly differentiated HCCs (40%). In contrast, arginase-1 and GPC-3 yielded higher sensitivity results in poorly differentiated HCCs (80%) and (70%), respectively. This is consistent with the known higher sensitivity of GPC-3 in poorly differentiated HCCs compared with well or moderately differentiated HCCs^[21,22]. Such finding is different from results of Ligato *et al*^[23] who observed that GPC-3 was expressed in 100% of HCC with nuclear grade I, 88.9 % with grade II and 62.5 % with grade III.

In the current study, arginase-1, HepPar-1, and GPC-3 were not completely specific for hepatic differentiation (90.6%), (87.5%), (93.8%), respectively. Also, the staining patterns of such biomarkers in adenocarcinomas in our analysis were different. Arginase-1 demonstrated a diffuse and strong reactivity in one case of breast

carcinoma and one case of pancreatic adenocarcinomas (25%) for which the liver is a frequent site of metastasis. These results support the possibility of sharing immunophenotype between HCC and pancreatic adenocarcinoma. A previous analysis of arginase-1 immunohistochemical expression in rats detected that arginase-1 was expressed at high levels in the liver and at moderate levels in the pancreas^[15]. Thus, it is not surprising that a subset of the pancreatic adenocarcinomas included in our study showed arginase-1 immunoreactivity. These findings are in agreement with the study of Fujiwara *et al*^[19]. The authors reported that arginase-1 is not entirely specific for hepatic differentiation, as immunoreactivity can be identified in adenocarcinomas particularly of pancreatic origin. In contrast, Timek *et al*^[18], and McKnight *et al*^[20], reported negativity of arginase-1 in all investigated cases of MC. Also, Fatima *et al*^[24], failed to demonstrate any significant correlation to prove the hypothesis that hepato-pancreatic precursor or stem cells might persist in the adult liver and pancreas and the possibility of sharing immunophenotype. Both HepPar-1 and GPC-3 lacked immunoreactivity in pancreatic adenocarcinomas in our analysis.

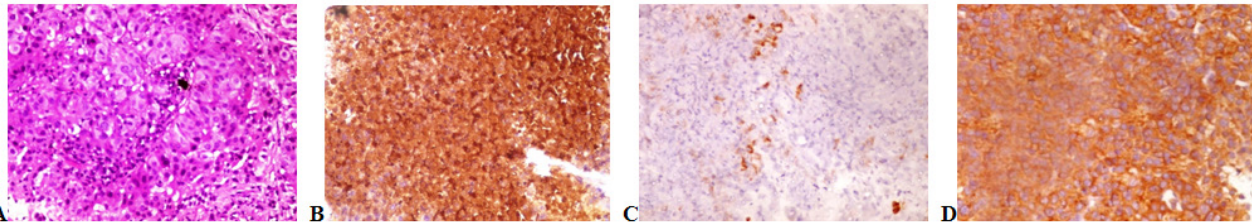


Figure 3 A: a case of poorly differentiated hepatocellular carcinoma (H&E x400); B: shows strong and diffuse arginase-1 staining (x400); C: shows focal HepPar-1 immunostaining (x400); D: shows strong and diffuse Glypican-3 immunostaining (x400).

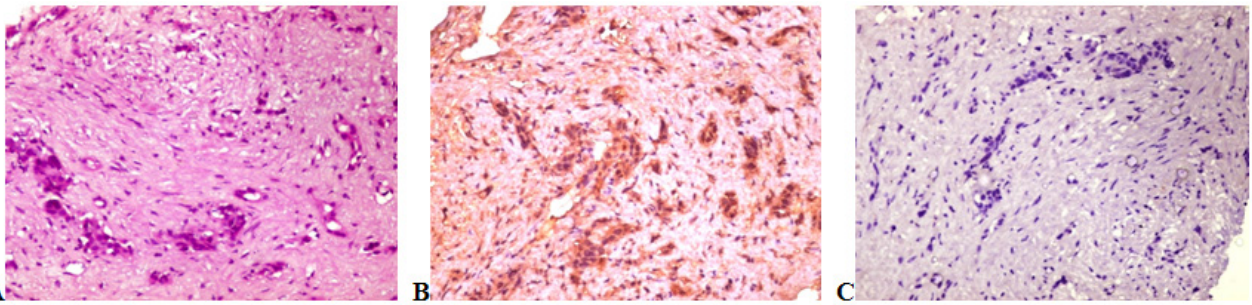


Figure 4 A: a case of intrahepatic cholangiocarcinoma (H&E x100); B: shows strong and diffuse arginase-1 immunostaining (x100); C: shows negative staining with Glypican-3 (x100).

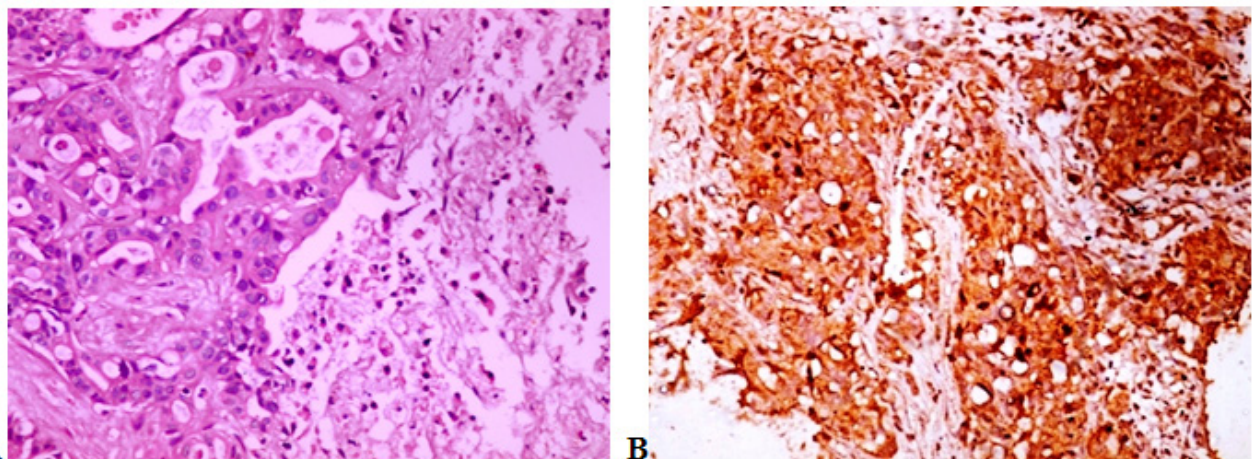


Figure 5 A: a case of a metastatic pancreatic adenocarcinoma to the liver (H & E, x400); B: shows strong and diffuse staining with arginase-1 (x400).

HepPar-1 was positive in pulmonary and colonic adenocarcinomas in this study, all of which were negative for arginase-1 and GPC-3. The positive immunostaining of HepPar-1 in three cases of MC (2 from colon and 1 from lung) was in concordance with the results of Yan *et al*^[4], who found HepPar-1 reactivity in 2 colonic adenomas, 8 colonic adenocarcinomas, 2 pulmonary adenocarcinomas, 1 chromophobe RCC and 9 gastric adenocarcinomas. Moreover, Timek *et al*^[18], stated that the expression of HepPar-1 in non-hepatocellular tumors is well documented in the literature and caution should be taken when using HepPar-1 to confirm a diagnosis of HCC. These findings disagree with other studies which showed complete negativity of HepPar-1 in all cases of MC^[25,26].

In our study, GPC-3 demonstrated a focal and strong staining in one case of colonic carcinoma and one case of breast carcinoma; HepPar-1 was positive in this case of colonic carcinoma and arginase-1 demonstrated diffuse strong staining in the same case of breast carcinoma. Out of four cases of CC, only one case was positive for arginase-1 and another one positive for HepPar-1. GPC-3 was typically negative in the four cases. The positivity of HepPar-1

in our study is in general agreement with that of Shiran *et al*^[26], and Wennerberg *et al*^[27]. Shiran *et al*^[26] reported that the presence of this occasional positivity should not be surprising considering the common progenitor cell of HCC and CC. Wennerberg *et al*^[27], also reported positivity in 2 out of 35 cases of CC (5.7%). A higher percentage of positivity in CC in our investigation (25%) could be due to the small sample size of such cases in our study. However, Fujiwara *et al*^[19], reported a negative immunoreactivity in all their investigated cases of CC for all biomarkers.

Our analysis of the different immunohistochemical marker combinations suggests that the combination of positive arginase-1 staining or positive HepPar-1 staining demonstrates sensitivity of (87.5%) and specificity (78.1%). In addition, the combined staining of both arginase-1 and HepPar-1 demonstrated high specificity (100%) with decreased sensitivity (71.9%). The high specificity of the arginase-1 and HepPar-1 combination was because immunohistochemical expression of arginase-1 and HepPar-1 in adenocarcinomas and cholangiocarcinoma in our analysis were mutually exclusive. Arginase-1-positive adenocarcinoma always

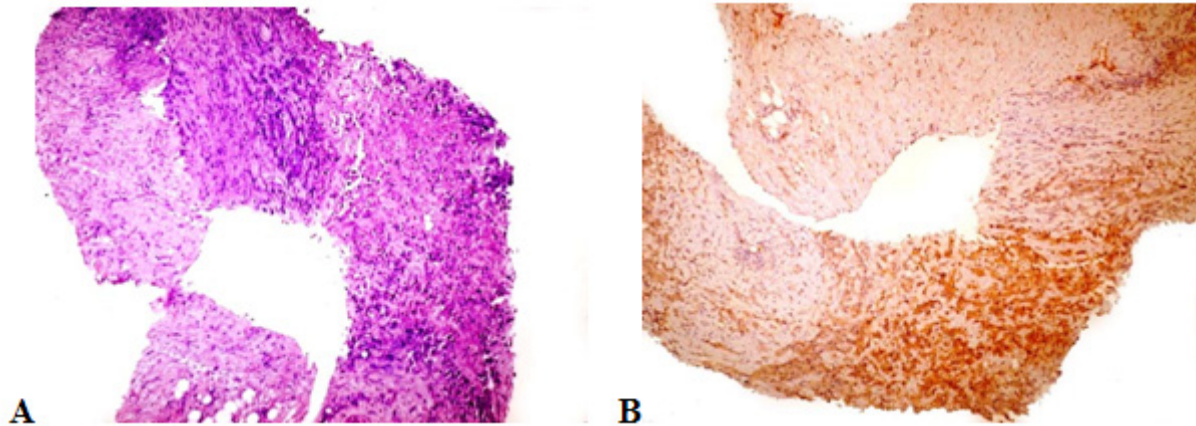


Figure 6 A: a case of a metastatic breast adenocarcinoma to the liver (H & E, x100); B: strong and diffuse staining with Glypican-3 (x100).

lacked HepPar-1 positivity and vice versa. On the other hand, the inclusion of glypican-3 in the panel of arginase-1 and HepPar-1 staining reduced sensitivity to 50% but specificity remained 100%. Although GPC-3 exhibited the lowest sensitivity of the three immunohistochemical stains evaluated in our study, it is highly specific for HCC. GPC-3 staining was not observed in any case of non-neoplastic liver tissue or CC and demonstrated positivity in only 7.1% of MC, suggesting its use for the distinction of HCC from non-neoplastic hepatocellular lesions and non-HCC. Although neither arginase-1 nor HepPar-1 IHC is helpful to differentiate HCC from benign liver lesions, both are extremely useful in the distinction of HCC from MC in the liver due to higher sensitivity.

CONCLUSION

This study confirmed that both arginase-1 and HepPar-1 are effective immunohistochemical biomarkers of hepatocellular differentiation. In addition, arginase-1 demonstrates superior sensitivity compared with GPC-3 and HepPar-1 in the diagnosis of HCC, whereas GPC-3 demonstrates superior specificity. Hence our data suggest that the use of Arginase-1, HepPar-1, and GPC-3 as a panel is highly efficacious in the differential diagnosis of HCC from non-HCC

CONFLICT OF INTERESTS

The authors declare no conflicts of interest in this work.

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