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The Diagnostic Role of Arginase-1, MOC-31, and CDX2 in the Differentiation of Hepatocellular Carcinoma, Cholangiocarcinoma, and Metastatic Colonic Carcinoma of the Liver

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Abstract

Background: Hepatocellular carcinoma (HCC) is the most common primary liver cancer. Pathologic differentiation between HCC from metastatic carcinoma and cholangiocarcinoma has critical therapeutic implications. However, it is occasionally challenging and sometimes requires immunohistochemical panels. Recently, Arginase-1, MOC-31, and CDX2 have been introduced for the differentiation of these tumors. This study was conducted to determine the value of expression of Arginase-1, MOC-31, and CDX2 in differentiating primary carcinoma of the liver from cholangiocarcinoma and metastatic adenocarcinoma to the liver.

Methods: 50 cases of HCC, 20 cases of metastatic colonic carcinoma to the liver, and 10 cases of cholangiocarcinoma were evaluated for immunohistochemical expression of Arginase-1, MOC-31, and CDX2.

Results: Arginase-1 was positive in 45 (90%) of HCC cases and negative in metastatic carcinoma and cholangiocarcinoma cases. MOC-31 was positive in 19 (95%) of metastatic colonic adenocarcinoma cases and 10 (100%) of cholangiocarcinoma cases, while it was negative in HCC cases. CDX2 was positive in 18 (90%) of metastatic carcinoma cases while it was negative in cholangiocarcinoma cases. The sensitivity of Arginase-1 for HCC, MOC-31 for MC, and CDX2 for metastatic colonic carcinoma in the studied groups was 95%, 100%, and 98%, respectively, whereas its specificity was 100%, 96.7%, and 60%, respectively. The difference of Arginase-1, MOC-31, and CDX2 expressions in HCC, cholangiocarcinoma, and metastatic colonic adenocarcinoma were statistically significant (P<0.001).

Conclusion: Our study revealed that Arginase-1, MOC-31, and CDX2 expression are suitable IHC markers in the differential diagnosis of HCC, cholangiocarcinoma, and metastatic colonic adenocarcinoma.

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Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver tumor in adults. Most of the malignant liver lesions are metastatic in origin rather than being induced by primary liver carcinoma.[1] Liver carcinoma is the most common cancer in Egypt, accounting for about 23.81% of all cancers.[2] Colorectal carcinoma is the most common primary tumor causing liver metastasis (35%).[3]

Cholangiocarcinomas (CCs) also can be challenging because they are usually adenocarcinomas. Therefore, it is difficult to differentiate CC from metastatic tumors or sometimes from less differentiated HCC.[4]

Immunohistochemistry plays a vital role in the differential diagnosis of liver tumors.[5]

Arginase is the enzyme that is responsible for the hydrolysis of arginine to ornithine and urea in the urea cycle. This enzyme exists in two isoforms; i.e., Arginase1 and Arginase 2. Arginase-1 shows high levels of expression within the liver.[6]

MOC-31 is a monoclonal antibody that recognizes the extracellular domain EpEX of epithelial cell adhesion molecule, which is a type-I transmembrane glycoprotein. It is expressed on the basolateral membrane in most normal epithelial tissues and is overexpressed in many human carcinomas.[7]

In the liver, MOC-31 is expressed in more than 90% of CC and metastatic adenocarcinoma (including colorectal, pancreas, stomach, lung, breast, and ovary) but it is negative or weakly positive in HCC.[8]

CDX2 is a member of the caudal-related homeobox gene family. It is involved in the processes of intestinal cell proliferation, differentiation, adhesion, and apoptosis.[9]

This study aimed to evaluate the IHC expression of Arginase-1, MOC-31 and CDX2 in differentiating primary carcinoma, especially poorly differentiated HCC, and CCs from metastatic adenocarcinoma in the liver and to correlate the expression of these markers and clinicopathological features.

Patients and methods

In our retrospective study, we included 80 sections from formalin fixed paraffin embedded tissue blocks that were collected from samples of 50 cases of hepatocellular carcinoma (core biopsies), 20 cases of metastatic colonic carcinoma to the liver, and 10 cases of cholangiocarcinoma. All cases were retrieved from the Pathology Department, Faculty of Medicine, Zagazig University, approved by the Ethical Committee during the period between 2013 and 2017. The clinical data, pathology reports, and hematoxylin and eosin (H&E) stained slides for the cases were reviewed to confirm the diagnosis. The histologic grade of HCC was established using the World Health Organization (WHO) criteria.[10] The patients with HCC were graded as 15 well differentiated, 26 moderately differentiated, and

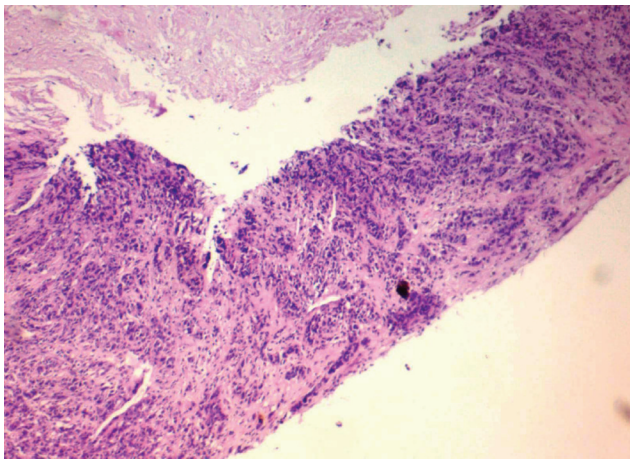

Figure 1. Hepatocellular carcinoma presenting malignant liver cells with hyperchromatic nuclei (H&E, Original magnification 40×).

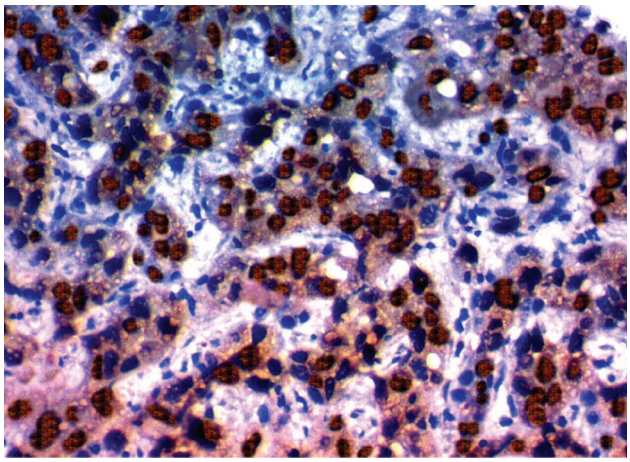

Figure 2. Hepatocellular carcinoma with strong arginase-1 staining (immunoperoxidase, original magnification 400×).

nine poorly differentiated. Cases of metastatic colonic carcinoma were proved using CK7 and CK20. Colonoscopy was done for those patients and confirmed primary colonic carcinoma by histopathology.

Compliance with Ethical Standards

This study was conducted following the statements of the Helsinki Declaration.

Immunohistochemical staining

A series of 4-µ thick sections of the formalin-fixed paraffin-embedded tissue blocks of the studied cases were investigated for the presence of a rabbit polyclonal anti-Arginase-1 antibody (H-52: sc 20150, Santa Cruz, Europe, dilution 1:200) and a mouse monoclonal anti-MOC 31 (clone MOC-31, 1 : 200 dilution; Biocare Medical, Concord, CA 94520 USA). CDX2 (CDX2 Std rabbit monoclonal antibody Cataloged (Cat.) was purchased from Thermo Scientific/Lab Vision Corporation, Fermont, USA, and clone: EPR2764; 0.09% sodium azide; Dilution 1:100). The binding site of primary antibodies was visualized by Dako EnVision ™ kit (Dako, Copenhagen, Denmark). Then, the sections were counterstained with Mayer's hematoxylin.

Evaluation of Immunohistochemical markers

Cytoplasmic and\or nuclear reactivity was considered as positive staining for arginase-1.[11] MOC-31 was expressed in a membranous pattern, and the tumor was deemed to be positive if more than 5% of its cells showed membranous staining.[12] CDX2 nuclear staining in tumor cells was considered if more than 6% of the cells were stained.[13]

Statistical analysis

Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 20. Quantitative variables were described using their means and standard deviations. Categorical variables were described using their absolute frequencies. Kolmogorov-Smirnov (distribution-type) and Levene (homogeneity of variances) tests were used to verify assumptions for use in parametric tests. To compare the means of two groups, we used the independent sample t-test when it was appropriate. Categorical data were compared using the Chi-square ($\chi 2$) test. Receiver operating characteristic (ROC) curve was used to assess the optimal cut-off value. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the data were calculated. The level of statistical significance was set at 5% ($P<0.05$). A highly significant difference was present if $P\leq 0.001$. On the other hand, $P>0.05$ was considered statistically nonsignificant (NS).

Results

Clinicopathological results

We enrolled 80 cases (50 female and 30 males), of which 50 cases were hepatocellular carcinoma,

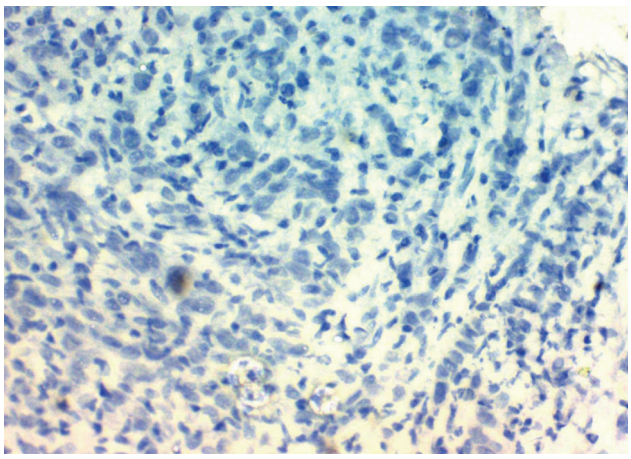

Figure 3. Hepatocellular carcinoma with negative MOC31 staining (immunoperoxidase, original magnification 200×).

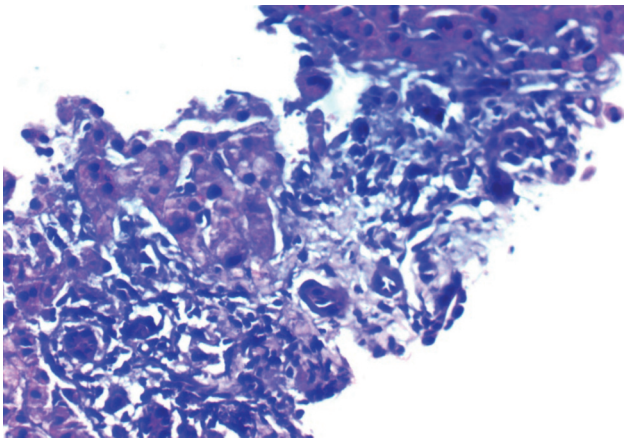

Figure 4. Cholangiocarcinoma is showing malignant glands surrounded by desmoplastic stroma. (H&E, original magnification 400×).

20 cases were metastatic carcinoma, and 10 were cholangiocarcinoma. All the demographic data are listed in table 1.

Immunohistochemical results

In our study, Arginase-1 is positive in 45 (90%) of cases of HCC and negative in metastatic carcinoma and cholangiocarcinoma cases (Figures 1 and 2). For MOC31, it is positive in 10 (100%) cases of cholangiocarcinoma and 19 cases (95%) of metastatic colonic adenocarcinoma cases, while it is negative in HCC (Figures 3, 4, 5 and 8). CDX2 is positive in 18 (90%) of metastatic colorectal carcinoma cases and is negative in cholangiocarcinoma cases. One case of HCC showed positive nuclear staining (Table 2) (Figures 6, 7 and 9).

The sensitivity of Arginase-1 for HCC in the studied group is 95%, whereas its specificity is 100%. The sensitivity of MOC-31 for AC in the studied group is 100%, while its specificity is 96.7%. The sensitivity of CDX2 for metastatic colonic carcinoma in the studied group is 98%, whereas its specificity is 60% (Table 3).

Based on the obtained results, there is a highly significant relation between Arginase-1 expression and associated liver cirrhosis and elevated Alfa-fetoprotein (*P*-value <0.0001 and <0.001 respectively). There is a highly significant relation between Arginase-1 expression and negative expression of both MOC-31 and CDX2 in cases of HCC (*P*-value <0.001) (Table 4). MOC-31 expression is inversely associated with both liver cirrhosis and elevated Alfa-fetoprotein (*P*-value <0.001). There is a highly significant relation between MOC-31 expression and negative expression of both Arginase-1 and CDX2 (Table 5). In this study, CDX2 expression shows a statistically significant association with high grade and HCC cases (*P*-value <0.001). CDX2 is inversely associated with liver cirrhosis (*P*-value <0.001) (Table 6)

Discussion

The most commonly encountered differential diagnostic challenge in the case of liver tumors is HCC versus intrahepatic cholangiocarcinoma or metastatic adenocarcinoma.[14] Some of these diagnostic challenges can be attributed to the following issues: a) The liver represents one of the three most common sites of metastasis, b) HCCs may show a variety of histologic patterns, mimicking a wide range of malignant tumors. Also, several metastatic tumors from the breast, pancreas, kidney, and adrenals may mimic the trabecular liver-like pattern of HCC. c) Cholangiocarcinoma and HCC often share overlapping morphologic appearances. d) The diagnosis process is complicated because pathologists are frequently asked to handle and diagnose tiny liver needle core biopsies.[15]

Arginase-1 has been described as a potential marker of hepatocellular differentiation.[11] Only a few studies have investigated arginase-1 expression in HCC, and most of these works have

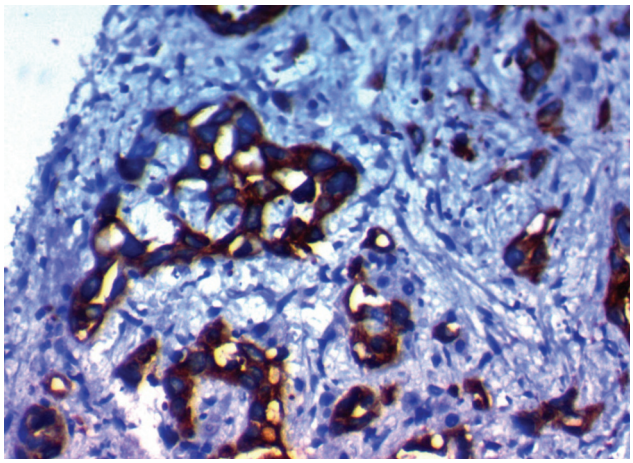

Figure 5. Cholangiocarcinoma with strong staining with MOC31 staining (immunoperoxidase, original magnification 400×).

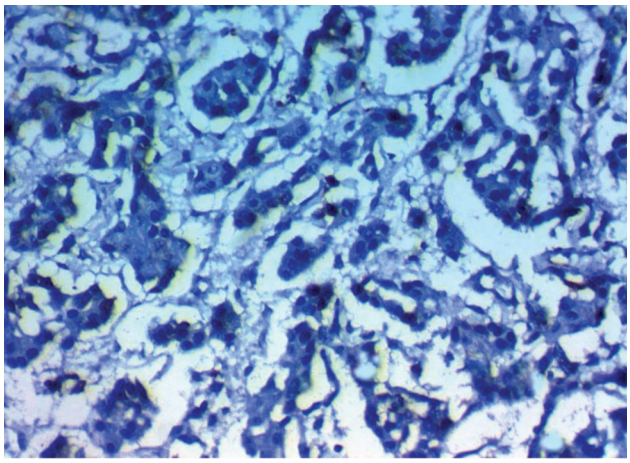

Figure 6. Cholangiocarcinoma with negative CDX2 (Staining immunoperoxidase, original magnification 400×).

Table 1. Clinicopathological features and immunohistochemical marker expression in the studied group.

Characteristics	Number	%
Age (year)		
Mean ± SD	54.24 ± 8.72	
Median (Range)	55 (39 – 70)	
Age group		
≤ 50 years	36	45%
> 50 years	44	55%
Gender		
Male	30	37.5%
Female	50	62.5%
Histopathology		
HCC	50	62.5%
Cholangiocarcinoma	10	12.5%
Metastatic lesion	20	25%
Tumor multiplicity		
Multiple foci	62	77.5%
Single focus	18	22.5%
Grade		
Grade I	21	26.2%
Grade II	38	47.5%
Grade III	21	26.2%
Alfa-fetoprotein		
Less than 20	15	18.8%
More than 20	65	81.2%
Associated with liver cirrhosis		
Absent	26	(32.5%)
Present	54	(61.5%)
MOC-31		
Negative	51	63.8%
Positive	29	36.2%
Arginase-1		
Negative	35	43.8%
Positive	45	56.2%
CDX2		
Negative	61	76.2%
Positive	19	23.8%

Continuous variables were expressed as mean ± SD and median (range). Categorical variables were expressed as number (percentage).

been performed on fine needle aspiration cytology,[16,17] with some variations in their interpretations as regards its sensitivity and specificity. Therefore, the primary purpose of the current research was to examine the immunohistochemical staining of Arginase-1 in cases of HCC, metastatic colonic carcinoma involving the liver, and cholangiocarcinoma as compared to MOC-31 and CDX2. Our study is an attempt to define its further diagnostic utility as a reliable positive

Table 2. Immunohistochemical expression of Arginase-1, MOC31, and CXD2 in the studied group.

	HCC (50)		Cholangiocarcinoma (10)		Metastatic carcinoma (20)	
	+ve N (%)	-ve N (%)	+ve N (%)	-ve N (%)	+ve N (%)	-ve N (%)
Arginase -1	45 (90%)	5 (10%)	0 (0%)	10 (100%)	0 (0%)	20 (100%)
MOC-31	0 (0%)	50 (100%)	10 (100%)	0 (0%)	19 (95%)	1 (5%)
CDX2	1 (2%)	49 (98%)	0 (0%)	10 (100%)	18 (90%)	2 (10%)

marker in differentiating these tumors to correlate these markers expression and clinicopathological factors.

Arginase-1 is positively expressed in HCC cases, and this was in contrast to negative staining for Argenase-1 in metastatic tumors, and cholangiocarcinoma studied cases. This finding is beneficial because one of the most common diagnostic challenges facing a pathologist examining liver focal lesion is distinguishing between poorly differentiated HCC from a metastasis, especially in the small biopsy specimen.

The results are consistent with a study[11] that reported Arginase-1 expression in 96% of studied HCC cases. The results are in line with study[18] that found Arginase-1 demonstrated positive immunoreactivity in 42 of 50 cases of HCC, with a 96% specificity of Arginase-1 for HCC diagnosis. Also, they reported negativity of Arginase-1 in all their MC cases.[16,17]

There is a highly significant relation between Arginase-1 expression and associated liver cirrhosis. According to the results of a study,[19] elevated Arginase-1 staining was associated with chronic HCV infection, and Arginase-1 expression was elevated in more than 75% of HCV-infected liver samples and (0% positive) in uninfected liver tissue. The authors suggested that the up-regulated expression of Arginase-1 was associated with HCV infected liver. They assumed that an essential part of the mechanism whereby HCV regulates hepatocellular growth and survival might be through altering arginine metabolism. However, further studies on a large scale are needed to confirm these observations.

In the present study, all 10 CC cases showed positive membranous immune-reactivity for MOC-31, with 100% sensitivity in the studied group. 95% of metastatic carcinoma cases showed membranous positivity for MOC-31, and the specificity of MOC-31 was 96.7%. A reasonably similar finding was observed in[20] a study that reported no MOC-31 staining in HCCs.[20] This result also is reported by other researchers.[21] The scientists[22] observed that 97% of metastatic adenocarcinoma was positive for MOC-31. The results of the current study are similar to those of a research[23] that found that the sensitivity of

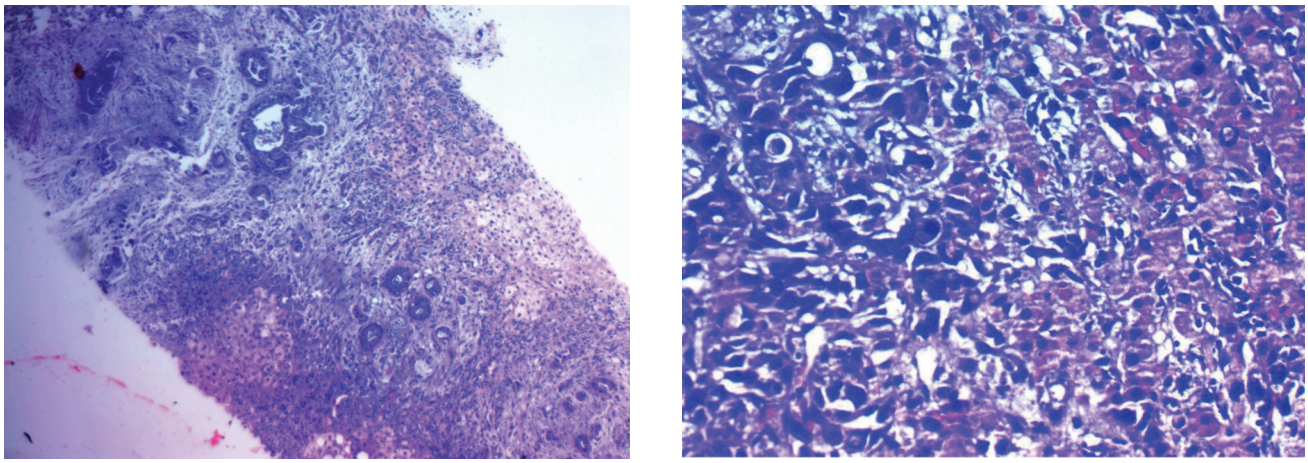

Figure 7. A) Metastatic colon adenocarcinoma to the liver (H&E, original magnification 40×), B) Metastatic colonic adenocarcinoma to the liver (H&E, original magnification 400×).

Table 3. Sensitivity and Specificity of Arginase-1, MOC-31, and CDX2 in the studied group.

Markers	Sensitivity% (95% CI)	Specificity% (95% CI)	PPV% (95% CI)	NPV% (95% CI)	Accuracy (95% CI)
Arginase -1	95% (78.1-96.67)	100% (88.4- 100)	100%	85.7% (72.3-93.2)	93.8% (86- 97.9)
MOC-31	100% (92.9-100)	96.7% (82.8-99.9)	98% (87.9-99.7)	100%	98.8% (93.2-100)
CDX 2	98% (89.4- 99.95)	60% (40.6-77.34)	80.3% (72.5-86.4)	94.7% (71.7- 99.2)	83.8% (73.8-91.1)

Chi-square test for trend; $P< 0.05$ is statistically significant.

MOC-31 for MC in the studied group was 97.2%, whereas its specificity was 90%. In contrast to these results, in another study,[24] MOC-31 expressions were found in 5 out of 42 (12%) HCC. Moreover, elsewhere,[25] it was found that 1 out of the 25 (4%) HCCs cases was positive for MOC-31. In this regard, they found a similar trend in favor of MOC-31 negativity in HCCs and MOC-31 positivity in metastatic adenocarcinoma, suggesting that MOC-31 is a valuable marker in the differential diagnosis. In this work, CDX2 was positive in 18 (90%) of metastatic colonic carcinoma. CDX2 was negative in cholangiocarcinoma cases. Only one case of HCC shows positive nuclear staining. These results are similar to those of Shah et al.[26]

According to a study,[27] CDX2 was expressed in 114 of 118 (97%) metastatic colorectal carcinoma cases. The researchers declared almost similar results by reporting a positive expression for CDX2 in 85.7% of metastatic carcinomas of the colon. The difference between positive expression of Arginase-1, MOC-31, and CDX2 in terms of HCC, CC, and metastatic adenocarcinoma was statistically significant.[28] The diagnostic importance of positive Arginase-1 and negative (MOC-31and CDX2) in differentiating between HCCs, CC, and metastatic colonic carcinoma showed a sensitivity of 95, 100, and 98%, specificity of 100, 96.7, and 60%, and accuracy of 93.8, 98.8, and 83.8%, respectively.

Differentiation of HCC from metastatic carcinoma has essential therapeutic implications. There are several treatment modalities for hepatocellular carcinoma. Correct classification of these tumors is critically important. The main reason for conducting this study is that HCC in Egypt is a serious national problem such that it accounts for about 23.81% of all cancers. To the best of our knowledge, a statistical analysis involving these different IHC profiles (Arginase-1, MOC-31, and CDX2) has not been carried out yet. Based on the results of this paper, employing these immunoprofiles can be of high significance

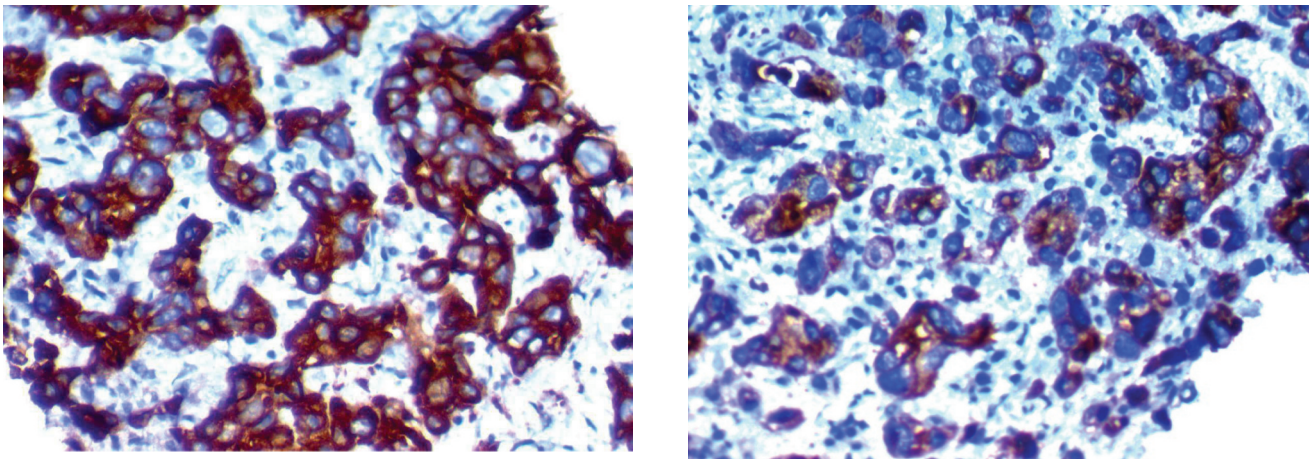

Figure 8. A) Metastatic colonic adenocarcinoma to the liver with strong MOC31 staining (immunoperoxidase, original magnification 400×), B) Metastatic colonic adenocarcinoma to the liver with strong MOC31 staining (immunoperoxidase, original magnification 400×).

Table 4. Relationship between clinicopathological features and Arginase-1 expression in the studied group.

Characteristics	All (N=80) No. (%)	Arginase-1 Negative (N=35) No. (%)	Positive (N=45) No. (%)	*P*-value
Age (years)				
Mean ± SD	54.24±8.72	54.73±9.2	49.50±3.93	0.567*
Median (Range)	55 (39-70)	54 (39-68)	55 (39-70)	
≤ 50 years	36 (45%)	16 (44.4%)	20 (55.6%)	0.91‡
> 50 years	44 (55%)	19 (43.2%)	25 (56.8%)	
Gender				0.684
Male	30 (37.5)	14 (47.6)	16 (52.4)	
Female	50 (62.5)	21 (42)	29 (58)	
Histopathology				
HCC	50 (62.5%)	5 (10%)	45 (90%)	<0.001‡
Cholangiocarcinoma	10 (12.5%)	10 (100%)	0 (0%)	
Metastatic lesion	20 (25%)	20 (100%)	0 (0%)	
Grade				
Grade I	21 (26.2%)	10 (47.6%)	11 (52.4%)	0.472§
Grade II	38 (47.5%)	14 (36.8%)	24 (63.2%)	
Grade III	21 (26.2%)	11(52.4%)	10 (47.6%)	
Associated liver cirrhosis				
Absent	26 (32.5%)	20 (76.9%)	6 (23.1%)	<0.0001‡
Present	54 (61.5%)	15 (27.8%)	39 (72.2%)	
Tumor multiplicity				
Multiple foci	62 (77.5%)	25 (40.3%)	37 (59.7%)	0.251‡
Single focus	18 (22.5%)	10 (55.6%)	8 (44.4%)	
Alfa-fetoprotein				
Less than 20	15 (18.8%)	15 (100%)	0 (0%)	<0.001‡
More than 20	65 (81.2%)	20 (30.8%)	45 (69.2%)	
MOC -31				
Negative	51 (43.8%)	6 (11.8%)	45 (88.2%)	<0.001‡
Positive	29 (56.2%)	29 (100%)	0 (0%)	
CDX 2				
Negative	61 (76.2%)	16 (26.2%)	45 (73.8%)	<0.001‡
Positive	19 (23.8%)	19 (100%)	0 (0%)	

Categorical variables were expressed as number (percentage).; Continuous variables were expressed as mean ± SD & median (range).; *Independent sample t-test.; ‡ Chi-square test§ Chi-square test for trend.; *P*< 0.05 is significant.

as diagnostic tools in the differential diagnosis of HCC, cholangiocarcinoma, and metastatic colonic adenocarcinoma. The choice of these markers is an essential issue in developing countries.

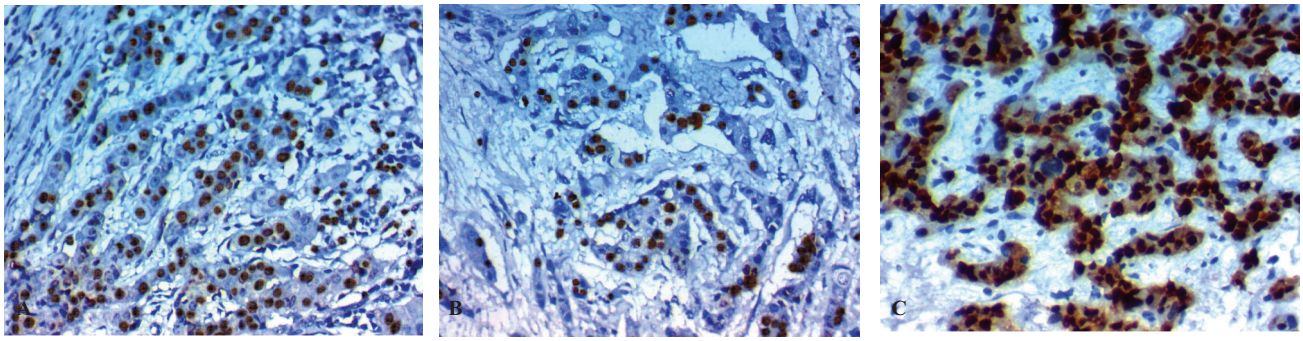

Figure 9. A) Metastatic colonic adenocarcinoma to the liver with strong CDX2 staining (immunoperoxidase, original magnification 400×), B) Metastatic colonic adenocarcinoma to the liver with strong CDX2 staining (immunoperoxidase, original magnification 400×), C) Metastatic colonic adenocarcinoma to the liver with strong CDX2 staining (immunoperoxidase, original magnification 400×).

Table 5. The relationship between clinicopathological features and MOC-31 expression in the studied group.

Characteristics	All (N=80) No. (%)	MOC-31 Negative (N=51) No. (%)	Positive (N=29) No. (%)	*P*-value
Age (years)				
Mean ± SD	54.24±8.72	54.33±9.29	54.07±7.76	0.897*
Median (Range)	55 (39-70)	55 (39-70)	55 (41-68)	
≤ 50 years	36 (45%)	22 (61.1%)	14 (38.9%)	<0.657‡
> 50 years	44 (55%)	29 (65.9%)	15 (34.1%)	
Gender				
Male	30 (37.5)	21 (70)	9 (30)	0.368
Female	50 (62.5)	30 (60)	20 (40)	
Histopathology				
HCC	50 (62.5%)	50 (100%)	0 (0%)	<0.001‡
Cholangiocarcinoma	10 (12.5%)	0 (28.6%)	10 (100%)	
Metastatic lesion	20 (25%)	1 (5%)	19 (95%)	
Grade				
Grade I	21 (26.2%)	15 (71.4%)	6 (28.6%)	0.196§
Grade II	38 (47.5%)	26 (68.4%)	12 (31.6%)	
Grade III	21 (26.2%)	10 (47.6%)	11 (52.4%)	
Associated with liver cirrhosis				
Absent	26 (32.5%)	6 (23.1%)	20 (76.9%)	<0.0001‡
Present	54 (61.5%)	45 (83.3%)	9 (16.7%)	
Tumor multiplicity				
Multiple foci	62 (77.5%)	42 (67.7%)	20 (32.3%)	0.168‡
Single focus	18 (22.5%)	10 (28.6%)	8 (17.8%)	
Alfa-fetoprotein				
Less than 20	15 (18.8%)	1 (6.7%)	14 (93.3%)	<0.001‡
More than 20	65 (81.2%)	50 (76.9%)	15 (23.1%)	
Arginase-1				
Negative	35 (63.8%)	6 (17.1%)	29 (82.9%)	<0.001‡
Positive	45 (36.2%)	45 (100%)	0 (0%)	
CDX 2				
Negative	61 (76.2%)	50 (82%)	11 (18%)	<0.001‡
Positive	19 (23.8%)	1 (5.3%)	18 (94.7%)	

Categorical variables were expressed as number (percentage).; Continuous variables were expressed as mean ± SD & median (range).; * Independent sample t-test ‡ Chi-square test. § Chi-square test for trend. P< 0.05 is significant.

The small number of cases may limit providing a reliable statistical diagnosis. Thus, this study has to be further extended to include a higher number of cases.

Conclusion

The present study showed that Arginase-1 immunostaining has a higher sensitivity and specificity for HCC diagnosis. Arginase-1 provides a potentially promising tool in distinguishing HCC from MC and CC. MOC31 may be used as a diagnostic marker for cholangiocarcinoma. CDX2 is mostly expressed in metastatic colonic carcinoma, so it is a useful marker for diagnosis. The combination of these markers has a role in the diagnosis of problematic cases.

Conflict of Interest

None declared.

Table 6. The relationship between clinicopathological features and CDX2 expression in the studied group.

Characteristics	All (N=80) No. (%)	CDX 2 Negative (N=61) No. (%)	Positive (N=19) No. (%)	*P*-value
Age (years)				
Mean ± SD	54.24±8.72	54.3±8.74	54.05±8.88	0.916*
Median (Range)	55 (39-70)	55 (39-70)	55 (40-68)	
≤ 50 years	36 (45%)	27 (75%)	9 (25%)	0.812‡
> 50 years	44 (55%)	34 (77.3%)	10 (22.7%)	
Gender				
Male	30 (37.5)	24 (80)	6 (20)	0.542
Female	50 (62.5)	37 (74)	13 (26)	
Histopathology				
HCC	50 (62.5%)	49 (98%)	1 (2%)	<0.001‡
Cholangiocarcinoma	10 (12.5%)	10 (100%)	0 (0%)	
Metastatic lesion	20 (25%)	2 (10%)	18 (90%)	
Grade				
Grade I	21 (26.2%)	20 (95.2%)	1 (4.8%)	0.001§
Grade II	38 (47.5%)	31 (81.6%)	7 (18.4%)	
Grade III	21 (26.2%)	10 (47.6%)	11 (52.4%)	
Associated liver cirrhosis				
Absent	26 (32.5%)	12 (46.2%)	14 (53.8%)	<0.001‡
Present	54 (61.5%)	49 (90.7%)	5 (9.3%)	
Tumor multiplicity				
Multiple foci	62 (77.5%)	46 (74.2%)	16 (25.8%)	0.422‡
Single focus	18 (22.5%)	15 (83.3%)	3 (16.7%)	
Alfa-fetoprotein				
Less than 20	15 (18.8%)	9 (60%)	6 (40%)	0.101‡
More than 20	65 (81.2%)	52 (80%)	13 (20%)	
Arginase-1				
Negative	35 (43.8%)	16 (45.7%)	19 (54.3%)	<0.001‡
Positive	45 (56.2%)	45 (100%)	0 (0%)	
MOC -31				
Negative	51 (63.8%)	50 (98%)	1 (2%)	<0.001‡
Positive	29 (36.2%)	11 (37.9%)	18 (62.1%)	

Categorical variables were expressed as number (percentage). Continuous variables were expressed as mean ± SD & median (range). * Independent sample t-test ‡ Chi-square test. § Chi-square test for trend. $P< 0.05$ is significant.

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