The role of arginase-1 and glypican-3 in differentiating hepatocellular carcinoma from metastatic carcinoma in fine-needle aspiration cytology

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Purpose **The aim of the study was to evaluate the role of the novel marker arginase-1 in differentiating hepatocellular carcinoma (HCC) from metastatic adenocarcinoma and compare the results with those for glypican-3.**

Patients and methods **This is a retrospective study including 124 cases with liver masses referred from the Radiology Department to the Cytology Unit, Pathology Department, NCI. The pathological diagnosis and/or radiological picture were the gold standard. From each cell block two slides were stained with anti-arginase-1 and glypican-3. Sensitivity, specificity, positive and negative predictive values, percentage of marker expression as well as intensity and distribution of both markers among different grades were evaluated, and comparison of these items between the two markers was made.**

Results **The sensitivity and specificity of arginase-1 were 96.1 and 95.7%, respectively, and those for glypican-3 were 90.9 and 91.5%, respectively. Arginase-1 was expressed in 36 (97.3%) moderately differentiated and 30 (93.8%) poorly differentiated HCC cases, whereas glypican-3 expression was detected in 34 (91.9%) moderately and 28 (87.5%) poorly differentiated HCCs, respectively. All cases of well-differentiated HCC showed strong and diffuse staining for arginase-1, compared with seven (87.5%) cases for glypican-3. In the moderately differentiated group, 26 (72.2%) cases exhibited strong and diffuse staining for arginase-1, and 10 (27.8%) showed strong focal staining for glypican-3; 5 (14.7%), 18 (53%) and 11 (34.4%) cases stained as strong and diffuse, strong but focal, and weak and focal, respectively. In poorly differentiated cases, 13 (43.3%) stained focally weak, whereas all cases showed focal weak reactivity for glypican-3.**

Conclusion **Arginase-1 demonstrated superior sensitivity and specificity compared with glypican-3: 95.7% of higher-grade HCC cases were positive for arginase-1, whereas 89.9% were reactive for glypican-3; 46% of cases showed strong and diffuse staining pattern for arginase-1 compared with 17.1% for glypican-3. *Egypt J Pathol* 00:000–000 © 2013 Egyptian Journal of Pathology.**

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Keywords: arginase-1, fine-needle aspiration cytology, glypican-3, hepatocellular carcinoma, ICC

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Introduction

Hepatocellular carcinoma (HCC) is the most common liver neoplasm. It is considered the sixth most common malignancy worldwide with an incidence of 626 000 cases and 598 000 deaths annually, making it the third most common cause of cancer-related death after lung and stomach cancer (Parkin *et al.*, 2005).

Although the highest liver cancer rates are found in East and Southeast Asia and sub-Saharan Africa, the incidence is increasing in other areas because of the increased incidence of chronic hepatitis C infection (El-Serag, 2001; Jemal *et al.*, 2011). In contrast, the liver is a common site for metastatic tumors, accounting for 25% of all metastases to solid organs (Crawford, 2004).

Because of its relatively low cost, rapid turnaround time, various advantages, and high accuracy, fine-needle aspiration cytology (FNAC) of the liver under ultrasound or computed tomography guidance has been used in the routine diagnosis of masses of the liver to establish a diagnosis for liver masses. It is a less-invasive method, with a complication rate lower than that of core needle biopsy (Soyuer *et al.*, 2003; Yang *et al.*, 2004).

Unfortunately, diagnostic pitfalls exist in the morphologic distinction of HCC from metastatic carcinoma, particularly in small biopsy material with limited sampling and in poorly differentiated tumors. Because a clear distinction between HCC and metastatic tumors in the liver is clinically important as it carries a significant impact on subsequent prognosis and therapeutic management (Chu *et al.*, 2002; Ozer *et al.*, 2008), the application of adjunct diagnostic tools, such as immunocytochemical staining, which can be selected on the basis of suspected diagnoses, is therefore sometimes essential for a definitive diagnosis (Saad *et al.*, 2004).

A number of diagnostically useful immunohistochemical (IHC) markers for identification of HCC in routine surgical pathology practice remains limited to hepatocyte paraffin antigen (HepPar-1), polyclonal carcinoembryonic antigen, CD10, and α-fetoprotein. However, the utility of each of these markers is limited either by suboptimal sensitivity or by difficulty in interpretation (Kakar *et al.*, 2007).

Few previous studies conducted on liver FNAC have used a panel of the three most effective markers, arginase-1 (Arg-1), HepPar-1, and glypican-3 (GPC3), to demonstrate their efficacy in liver FNAC in differentiating HCC from metastatic adenocarcinomas. Most of these studies showed that, although HepPar-1 is sensitive and specific in the distinction between primary and metastatic liver tumors, its sensitivity drops in poorly differentiated

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HCC. Furthermore, HepPar-1 positivity is not entirely specific for tumors of hepatocellular origin (Wee, 2005).

Arg-1, a newly described marker in some studies, is a key urea cycle metalloenzyme, an enzyme involved in the hydrolysis of arginine to ornithine and urea, and was recently recognized as a sensitive and specific marker for benign and malignant hepatocytes (Yan *et al.*, 2010). In sections of normal liver and HCC, antiArg-1 produced strong, diffuse cytoplasmic reactivity in all hepatocytes throughout the lobule. There is no reactivity in bile duct epithelial cells, sinusoidal endothelial cells, Kupffer cells, or vascular endothelial cells (Multhaupt *et al.*, 1987).

In the literature, Arg-1 was proved to be a useful diagnostic marker in the differentiation of HCC from metastatic carcinoma. The usefulness of Arg-1 as an IHC marker of hepatocellular differentiation has been studied in surgical specimens. Previous IHC studies examining the expression of Arg-1 showed that the sensitivity and specificity of this marker for HCC reached up to 96.0 and 100%, respectively (McKnight *et al.*, 2012). However, there were only few reports that studied the usefulness of Arg-1 on FNA specimens. This is an important point because the diagnostic challenge of determining HCC from other malignancies frequently involves small biopsy or FNA specimens (Taylor and Haque, 2011).

GPC3 is a member of the glypican family of heparan sulfate proteoglycans. It is a cell-bound protein that attaches to the cell surface by a glycosyl-phosphatidylinositol anchor and plays an important role in cell growth and differentiation (Filmus and Selleck, 2001). Expression of GPC3 has been observed in some embryonic tissue, including the liver, but not in the corresponding normal adult tissue. Overexpression of this protein has been observed in HCC cells. Several recent studies have identified that GPC3, when used as a single IHC marker, or as part of a panel with other markers, may be very helpful in differentiating HCC from metastatic neoplasms to the liver. These studies, performed on formalin-fixed paraffin-embedded tissues, have demonstrated that GPC3 is expressed in the majority of cases of HCC with a sensitivity ranging from 72 to 90% and specificity between 96 and 100%. (Libbrecht *et al.*, 2006; Wang *et al.*, 2006).

In the literature, Arg-1 demonstrated superior sensitivity compared with GPC3 in the diagnosis of HCC on FNAC. In addition, Arg-1 exhibits more diffuse staining in HCC compared with GPC3, making interpretation easier in limited FNAB samples (McKnight *et al.*, 2012).

A large number of previous studies have compared the expression of both Arg-1 and HepPar-1 and have demonstrated superior sensitivity and specificity for Arg-1 compared with HepPar-1 (Fan *et al.*, 2003; Fu *et al.*, 2004; Yang *et al.*, 2004; Yan *et al.*, 2010; Radwan and Ahmed, 2012). However, to our knowledge, there are very few reports in the literature on a comparison between the expression of Arg-1 and that of GPC3, especially in liver FNAC, although since then GPC3 has been shown to be a sensitive and specific marker that differentiates HCC from metastatic carcinoma to the liver (Wee, 2005; Kandil *et al.*, 2007; Anatelli *et al.*, 2008; Ligato *et al.*, 2008; Wang *et al.*, 2008; Shirakawa *et al.*, 2009; Timek *et al.*, 2012).

The present study was postulated to compare the results of the novel marker Arg-1 with that of GPC3 in liver FNAC.

Aim of the work

In this study we examined the expression of the novel marker Arg-1 in HCC to detect its usefulness as a marker in differentiating HCC from metastatic carcinoma and compare the results with those of GPC3.

Patients and methods

The present study was conducted on 124 patients who presented with liver masses, referred from the Radiology department to the Cytology unit, Pathology department, National Cancer Institute, Cairo University, during the period from January 2010 to June 2012. Patients' files were reviewed and data on age, sex, site, number and size of lesions, radiological information, tumor marker serum level, and any other relevant data were recorded.

The material of this study was obtained from archives, including cell blocks; the patients were unknown to us, and hence no consent was taken for the work.

FNAC was taken from liver masses under ultrasound guidance in the radiology department using a 22 G needle; six slides of smear and material for cell blocks were prepared for each case. The smears were immediately fixed in 95% ethyl alcohol. Both slides and cell blocks were sent to our unit. The smears were left in alcohol for 30 min at room temperature and then stained using modified Papanicolaou stains (Gill *et al.*, 1974).

Each slide was evaluated for cellular adequacy; cases were diagnosed into either HCC or metastatic carcinoma according to well-established morphologic criteria related to the pattern of cellular arrangement, cellular and nuclear details, and background characteristics (Orell *et al.*, 2005). In HCC cases the malignant cells were arranged in trabecular pattern with small capillaries transgressing tumor cells; the cells appeared hepatocyte-like, with centrally located nuclei, prominent nucleoli, and intranuclear inclusion; binucleation or multinucleation was observed in some cases; the cytoplasm was abundantly vacuolated, and some contained bile; the background showed dissociated bare malignant hepatocyte nuclei, and necrosis was present in some cases.

In metastatic cases the malignant cells were arranged in cluster, acini, or sheets, with no capillaries transgressing tumor cells. The cells appeared glandular with high nuclear cytoplasmin ratio. The nuclei were either centrally or peripherally located, without multinucleation or inclusions. The nucleoli ranged from inconspicuous to prominent, the cytoplasm was abundantly vacuolated, and did not contain bile. The malignant groups were mixed with that of benign hepatocytes in some cases; the background showed either necrosis or mucin. When the cytomorphological features belonging to HCC and metastatic carcinoma overlapped, the cases were diagnosed as adenocarcinoma of primary versus metastatic origin.

The HCC cases were divided into three groups: well differentiated, moderately differentiated, and poorly differentiated HCC.

Cases showing hypocellularity, hemorrhage, or bad quality were considered inadequate and excluded from the study. All cases included in the present study had either confirmatory core biopsy, or certain radiologic criteria and tumor marker serum level favoring the diagnosis of either tumor.

For each case, one cell block and one hematoxylin and eosin slide from each block were prepared for evaluation of adequacy. An additional two sections were prepared on positively charged glass slides and stained with anti-Arg-1 using ready-to-use rabbit monoclonal antibodies from Cell Marque (Rocklin, California, USA) (clone: SP156), and anti-GPC3, the ready-to-use mouse monoclonal antibody from Cell Marque (clone 1G12), using the avidin biotin peroxidase technique; the reaction was detected using diaminobenzidine with hydrogen peroxide. All slides were counterstained with hematoxylin. Appropriate positive control (histologic section of HCC for Arg-1 and GPC3) and negative controls (by substituting PBS for the primary antibody) were used.

Results of immunocytochemical staining were evaluated and compared with the results of core biopsy and radiological findings. Cases were considered positive for Arg-1 and GPC3 when either showed cytoplasmic or cytoplasmic plus nuclear reactivity in more than 5% of malignant cells. Staining intensity (weak or strong) and distribution of both markers (focal or diffuse) were evaluated. Staining was considered focal if fewer than 25% of cells were positive, and diffuse if more than 25% were positive

For each marker, sensitivity, specificity, positive predictive value, negative predictive value, concordance, and discordance rate were calculated. The percentage of marker expression was evaluated as well as the intensity and distribution of both markers among different grades. A comparison of these items between the two markers was then made.

Results

The present study included 124 cases with liver masses, 79 patients were male and 45 were female, with a male-to-female ratio of 1.7 : 1. The age of the patients ranged from 39 to 86 years, with a median age of 60 years. Seventy-eight patients presented with a single hepatic focal lesion, whereas 46 presented with multiple focal lesions.

Fifty-eight (46.8%) cases were diagnosed cytologically as HCC, including 8 (13.7%) cases classified as well-differentiated HCC, 37 (63.8%) as moderately differentiated, and 13 (22.4%) as poorly differentiated HCC. Thirty-six (29%) cases were diagnosed as metastatic adenocarcinoma, [comprising 15 cases (41.7%) of breast carcinoma, 12 (33.3%) of colonic carcinoma, 7 (19.5%) of lung carcinoma, and 2 cases (5.5%) of carcinoma of unknown primary origin]. The remaining 30 (24.2%) cases showed overlapping morphologic features between HCC and metastatic adenocarcinoma and were diagnosed as poorly differentiated adenocarcinoma of primary versus metastatic origin (Table 1).

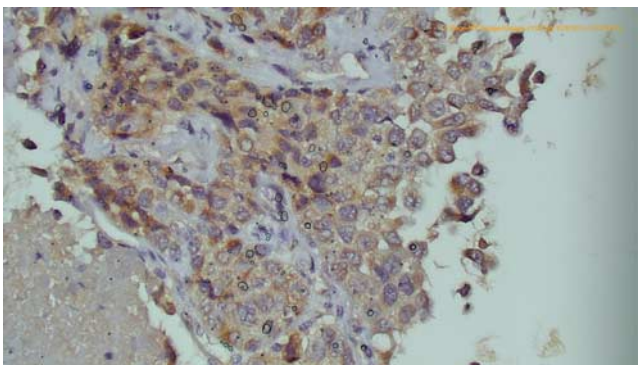
By comparing the results of cytomorphologic diagnosis with that of core biopsy and/or radiological criteria used to differentiate HCC from metastatic adenocarcinoma, all cases of HCC and metastatic carcinoma were confirmed. Of the 30 cases diagnosed cytologically as poorly differentiated carcinoma, either primary or metastatic, 19 (63.3%) were proved to be HCC, whereas the remaining 11 (36.7%) were that of metastatic adenocarcinoma origin. Thus, the total number of HCC cases was 77 (62.1%) and that of metastatic carcinoma was 47 (37.9%).

When immunocytochemistry for both markers was applied, it was found that Arg-1 was positive in 74 (96.1%) cases (Fig. 1) and negative in 3 (3.9%) HCC cases, whereas it was negative in 45 (95.7%) cases and positive in 2 (4.3%) cases of metastatic denocarcinoma (including one case of metastatic breast carcinoma and one case of pancreatic carcinoma). In contrast, glypican-3 was positive in 70 (90.9%) (Fig. 2) and negative in 7 (9.1%) cases of HCC. It was negative in 43 (91.5%) cases and positive in 4 (8.5%) cases of metastatic carcinoma (including two cases of lung carcinoma, one case of breast, and one case of gastrointestinal tract carcinoma) (Table 2). Thus, the sensitivity, specificity, positive predictive value, and negative predictive value for Arg-1 were 96.1, 95.7, 97.4, and 93.7%, respectively, and that for GPC3 were 90.9, 91.5, 94.6, and 86%, respectively. The concordance rate for Arg-1 was 96% and discordance was 4% and that for GPC3 were 91.1 and 8.9%, respectively.

Table 1 Distribution of the cases according to cytologic diagnosis

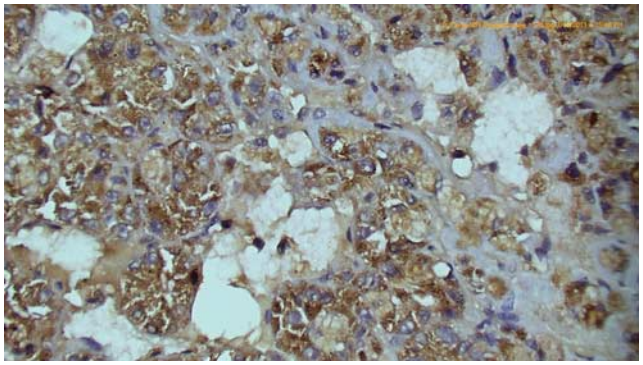
Cytologic diagnosis	*N* (%)
Hepatocellular carcinoma	58 (46.8)
Well differentiated	8 (13.8)
Moderately differentiated	37 (63.8)
Poorly differentiated	13 (22.4)
Metastatic carcinoma	36 (29)
Mammary carcinoma	15 (41.7)
Colorectal carcinoma	12 (33.3)
Pulmonary carcinoma	7 (19.5)
Carcinoma of unknown primary	2 (5.5)
Adenocarcinoma either primary or metastatic	30 (24.2)

Fig. 1



Cell block of a case of HCC showing positive cytoplasmic immunoreactivity for arginase-1 (× 400). HCC, hepatocellular carcinoma.

Fig. 2



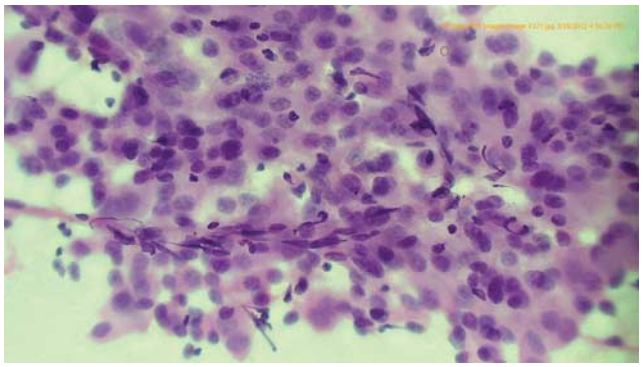
Cell block of a case of HCC showing positive cytoplasmin staining for glypican-3 (× 400). HCC, hepatocellular carcinoma.

Table 2 Arginase-1 and glypican-3 immunocytochemical expression

		Arginase-1 [*N* (%)]		Glypican-3 [*N* (%)]	
Diagnosis	Number	Positive	Negative	Positive	Negative
HCC	77	74 (96.1)	3 (3.9)	70 (90.9)	7 (9.1)
Metastatic carcinoma	47	2 (4.3)	45 (95.7)	4 (8.5)	43 (91.5)
Total	124	76	48	74	50

HCC, hepatocellular carcinoma.

Fig. 3



FNAC of a case of well-differentiated hepatocellular carcinoma showing a sheet of atypical hepatocytes, with mild degree of anaplasia, abundant eosinoplilic cytoplasm, and endothelial entrapment (Pap, × 400). FNAC, fine-needle aspiration cytology.

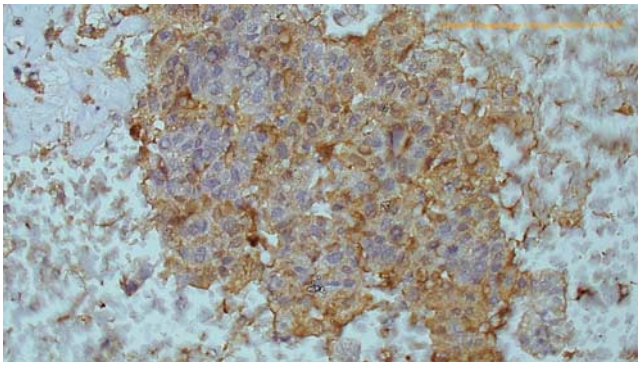
Of the 77 HCC cases included in our study, 8 (10.4%) were classified as well-differentiated HCC (Fig. 3), 37 (48.1%) as moderately differentiated, and 32 (41.5%) as poorly differentiated HCC, including 13 (40.6%) cases diagnosed cytologically and 19 (59.4%) cases of adenocarcinoma of either primary or metastatic origin that were confirmed after correlation with pathologic and/or radiologic assessment. GPC3 was expressed in all (100%), 34 (91.9%), and 28 (87.5%) cases of well, moderately, and poorly differentiated HCC, respectively, whereas Arg-1 was expressed in all (100%), 36 (97.3%), and 30 (93.8%) cases of well-differentiated, moderately differentiated, and poorly differentiated HCC, respectively (Table 3).

Table 3 Arginase-1 and glypican-3 expression in different grades of hepatocellular carcinoma

		Marker expression [*N* (%)]	
HCC	Number	Arginase-1	Glypican-3
Well differentiated	8	8 (100)	8 (100)
Moderately differentiated	37	36 (97.3)	34 (91.9)
Poorly differentiated	32	30 (93.8)	28 (87.5)
Total	77	74	70

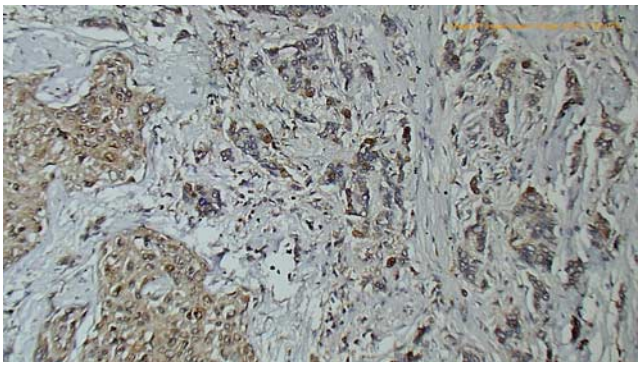
HCC, hepatocellular carcinoma.

Fig. 4



Cell block from a case of well-differentiated HCC showing strong diffuse cytoplasmic immunoreactivity for arginase-1 (× 400). HCC, hepatocellular carcinoma.

Fig. 5



Cell block from a case of moderately differentiated HCC showing strong but focal cytoplasmic immunoreactivity for arginase-1 (× 200). HCC, hepatocellular carcinoma.

For Arg-1, all eight cases of well-differentiated HCC showed strong and diffuse staining (Fig. 4). In the moderately differentiated group, 26 (72.2%) of 36 cases showed strong and diffuse staining, and the remaining 10 cases (27.8%) showed a strong but focal pattern of staining (Fig. 5). In the poorly differentiated group 17 (56.7%) showed strong and focal staining and the remaining 13 (43.3%) stained focally weak. In contrast, seven (87.5%) cases in the well-differentiated group reacted strongly and diffusely positive for GPC3 (Fig. 6), whereas the remaining case (12.5%) showed strong but focal staining. Moderately differentiated cases of HCC showed GPC3 immunostaining as follows: 5 (14.7%), 18 (53%), and 11 (34.4%)

cases showed strong and diffuse, strong but focal (Fig. 7), and weak and focal staining, respectively. All poorly differentiated cases exhibited focal and weak cytoplasmic reactivity (Table 4).

Discussion

A diagnosis of HCC at either end of the differentiation spectrum can represent a diagnostic challenge for cytopathologists in FNA material. Moreover, the treatment and prognosis of HCC and metastatic carcinoma are significantly different (Saad *et al.*, 2004). This highlights the need for sensitive and specific ICC markers for differentiation between HCC and metastatic adenocarcinoma (Wee, 2006).

A number of IHC markers of hepatocytes, including polyclonal carcinoembryonic antigen, CD10, α-fetoprotein, HepPar-1, and GPC3 have been and continue to be thoroughly studied in numerous studies, including tissue microarray-based studies, as well as in cytologic specimens (Saad *et al.*, 2004; Wang *et al.*, 2006). However, the application of such markers in many previous studies has shown significant diagnostic limitations (Wee, 2006; Kakar *et al.*, 2007).

A recent literature characterized a new IHC marker, Arg-1, as a potential marker of hepatocellular differentiation in both surgical pathology and cytopathology. Arg-1 has been described as the most sensitive and specific marker for diagnosing HCC, especially when compared with HepPar-1, GPC3, and other markers (Yan *et al.*, 2010).

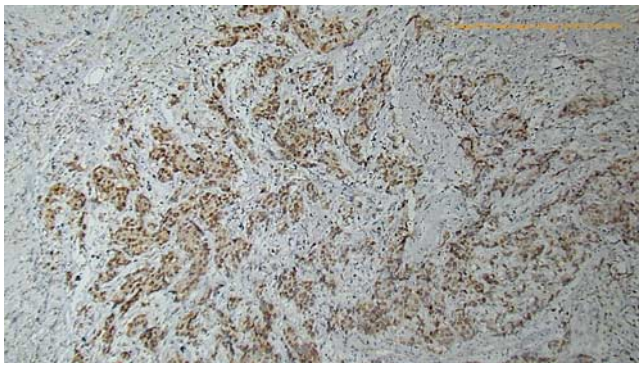
GPC3, a gene located on Xq26.1, has been studied extensively by many authors and is well established as a sensitive and specific IHC marker for HCC (Wang *et al.*, 2006). GPC3 was chosen in our study because there were very few reports in the literature that compared the results of Arg-1 as a marker of hepatocyte differentiation with that of GPC3.

The present work aimed at detecting the ability of the novel antibody Arg-1 as a marker of HCC, testing its usefulness to differentiate HCC from metastatic carcinoma to the liver on FNA samples and comparing the results with those of GPC3.

In the current study, Arg-1 was expressed in 74/77 (96.1%) cases of HCC, whereas it was negative in 45/47 (95.7%) cases of metastatic carcinoma; thus it showed a sensitivity and specificity of 96.1 and 95.7%, respectively. Our results support the findings observed by the previous studies conducted by Yan *et al.* (2010) and Timek *et al.* (2012) in which Arg-1 achieved a sensitivity of 96 and 94%, respectively. In contrast, our figures were higher than those found by Fujiwara *et al.* (2012), McKnight *et al.* (2012), and Radwan and Ahmed (2012), in which the sensitivities were 81, 84.1, and 84%, respectively.

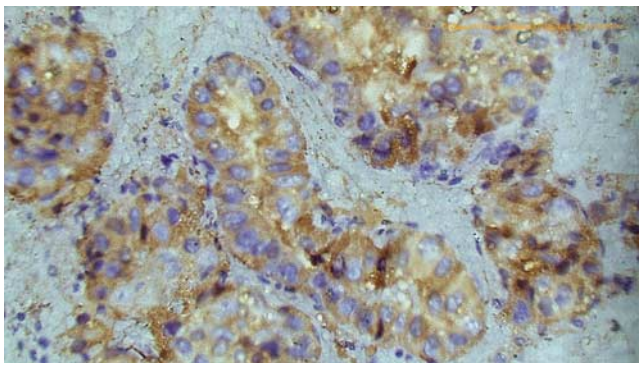
In the literature, Arg-1 achieved a specificity ranging from 87 to 100% (Yan *et al.*, 2010; Fujiwara *et al.*, 2012; McKnight *et al.*, 2012; Radwan and Ahmed, 2012; Timek *et al.*, 2012). The specificity for Arg-1 detected in the current work (95.7%) agreed with the values observed in previous studies.

Fig. 6



Cell block from a case of well-differentiated HCC showing strong diffuse cytoplasmic immunoreactivity for glypican-3 (× 200).
HCC, hepatocellular carcinoma.

Fig. 7



Cell block from a case of moderately differentiated HCC showing strong but focal cytoplasmic immunoreactivity for glypican-3 (× 400).
HCC, hepatocellular carcinoma.

Table 4 Pattern of arginase-1 and glypican-3 immunostaining in different grades of hepatocellular carcinoma cases

	Arginase-1 [*N* (%)]			Glypican-3 [*N* (%)]		
Pattern of staining	Strong and diffuse	Strong and focal	Weak and focal	Strong and diffuse	Strong and focal	Weak and focal
Well-differentiated HCC	8 (100)	–	–	7 (87.5)	1 (12.5)	–
Moderately differentiated HCC	26 (72.2)	10 (27.8)	–	5 (14.7)	18 (53)	11 (34.4)
Poorly differentiated HCC	–	17 (56.7)	13 (43.3)	–	–	28 (100)

HCC, hepatocellular carcinoma.

In contrast, GPC3 expression was detected in 70 of 77 (90.9%) cases of HCC and was negative in 43 of 47 (91.5%) cases of metastatic carcinoma, giving a sensitivity of 90.9% and a specificity of 91.5%.

The sensitivity of GPC3 achieved in the current study was similar to that observed by Kandil *et al.* (2007), who achieved a sensitivity of 90% for GPC3 in their study. Our results were higher than those observed in other studies in which the range of Glypican sensitivities ranged from 49 to 88% (Capurro *et al.*, 2005; Yamauchi *et al.*, 2005; Libbrecht *et al.*, 2006; Di Tommaso *et al.*, 2007; Anatelli *et al.*, 2008; Coston *et al.*, 2008; Ligato *et al.*, 2008; Wang *et al.*, 2008; Nassar *et al.*, 2009; Shirakawa *et al.*, 2009; Fujiwara *et al.*, 2012; McKnight *et al.*, 2012; Timek *et al.*, 2012).

Our figures regarding the specificity for GPC3 agreed with that observed in previous studies, in which it ranged from 89 to 100% (Capurro *et al.*, 2005; Yamauchi *et al.*, 2005; Libbrecht *et al.*, 2006; Kandil *et al.*, 2007; Coston *et al.*, 2008; Ligato *et al.*, 2008; Wang *et al.*, 2008; Nassar *et al.*, 2009; Shirakawa *et al.*, 2009; Fujiwara *et al.*, 2012; McKnight *et al.*, 2012; Timek *et al.*, 2012).

The current work showed that Arg-1 has superior sensitivity and specificity compared with GPC3 in differentiating HCC from metastatic adenocarcinoma. Similar to our findings, Fujiwara *et al.* (2012) and McKnight *et al.* (2012) have found a better sensitivity for Arg-1 compared with GPC3. In contrast, Fujiwara *et al.* (2012) failed to find a superior specificity for Arg-1, as its immunoreactivity was identified in adenocarcinoma from different organs. However, McKnight *et al.* (2012) reported a similar specificity for both markers.

In the current work, Arg-1 was better expressed in well-differentiated than in poorly differentiated HCC, as it was expressed in all cases (100%) of well-differentiated HCC cases, in 97.3% of moderately differentiated cases, and in 93.8% of poorly differentiated cases. In a similar manner, Yan *et al.* (2010) have noticed a better marker expression in lower-grade tumors than in higher-grade ones, where it was expressed in 100% of well-differentiated cases, in 96.2% in moderately differentiated cases, and in 85.7% of poorly differentiated cases. Radwan and Ahmed (2012) also demonstrated a better Arg-1 expression in lower-grade tumors where it was expressed in 100% of well-differentiated, 90% of moderately differentiated, and 44.4% of poorly differentiated HCCs. The current study failed to find a better sensitivity for Arg-1 in high-grade HCC, similar to our findings; Timek *et al.* (2012) failed to find such a relation.

This may be because of the small sample size of the cytologic specimens in the moderately to poorly differentiated HCC category, the limited number of cells in each case, and patchy/focal staining for Arg-1 in higher-grade HCC.

Similar to Arg-1, GPC3 was better expressed in well-differentiated HCC cases included in the present work, as it was expressed in all (100%), 34 (91.9%), and 28 (87.5%) well, moderately, and poorly differentiated HCC cases, respectively. Our findings were similar to those observed by Timek *et al.* (2012) who demonstrated more frequent positive staining for GPC3 in well to moderately differentiated HCCs than in higher-grade tumors. Ligato *et al.* (2008) also noticed decreasing GPC3 expression with increasing tumor grade. In contrast, Di Tommaso *et al.* (2007), Wang *et al.* (2008), and Shirakawa *et al.* (2009) demonstrated better expression of GPC3 in moderately and poorly differentiated cases, compared with well-differentiated ones.

In contrast to the present work that has found a relation between GPC3 expression and degree of differentiation, many previous studies failed to find such a relation (Yamauchi *et al.*, 2005; Libbrecht *et al.*, 2006; Kandil *et al.*, 2007; Anatelli *et al.*, 2008).

The present work showed that Arg-1 demonstrated better sensitivities among the moderately and poorly differentiated HCC cases compared with GPC3: 66/69 (95.7%) cases were reactive for Arg-1 compared with 62/69 (89.9%) cases for GPC3. Fujiwara *et al.* (2012) disagreed with our results as in their study the same numbers of moderately and poorly differentiated HCC cases (43%) were immunoreactive for both Arg-1 and GPC3.

Regarding the intensity and distribution of staining of HCC cases for Arg-1, all well-differentiated HCC cases a showed strong and diffuse staining pattern; most cases in the moderately differentiated group [26 out of 36 (72.2%)] showed a strong and diffuse staining pattern and the remaining 10 cases (27.8%) showed strong but focal pattern of staining, whereas in the poorly differentiated group 17 (56.7%) showed strong focal staining and the remaining 13 (43.3%) cases showed weak focal staining.

Similar to our results, Radwan and Ahmed (2012) demonstrated strong and diffuse staining for Arg-1 in all well-differentiated cases, and in 70.4% of moderately differentiated cases, whereas in the poorly differentiated group 50% of cases showed strong and diffuse staining and 50% showed weak and focal staining. Similarly, Yan *et al.* (2012) noticed that Arg-1 exhibited strong and diffuse staining in better differentiated tumors, where such staining pattern was present in 100, 73.6, and 53.6% of well, moderately, and poorly differentiated HCCs, respectively.

In contrast, 7/8 (87.5%) well-differentiated cases demonstrated diffuse and strong pattern of staining for GPC3; among the moderately differentiated HCC cases, 18 (53%) showed strong and focal staining pattern, followed by 11 (34.4%) cases with weak and focal staining, and 5 cases (14.7%) with strong diffuse reactivity. All poorly differentiated cases exhibited focal and weak cytoplasmic reactivity.

In contrast to our results that showed a strong and diffuse staining pattern only in 17.1% of GPC3-positive HCC cases, Kandil *et al.* (2007) found that most (90%) HCC cases showed a strong diffuse staining pattern for GPC3 expression. Similarly, Yamauchi *et al.* (2005)

reported strong diffuse GPC3 staining in 84% of HCCs, whereas Anatelli *et al.* (2008) found that about half of their cases (56%) showed diffuse immunoreactivity. These differences can be explained by using different clones of the antibody, different numbers of cases, and different marker expressions among different degrees of HCC differentiation.

In the present work, all cases in the well-differentiated group stained diffusely strong for Arg-1 (100%), compared with 87.5% for GPC3. Similarly, in moderately differentiated cases, 72.2% stained diffusely strong for Arg-1 compared with 14.7% for GPC3, and 27.8% of cases stained focally strong for Arg-1 compared with 53% for GPC3. In poorly differentiated cases, 56.7% stained focally strong and only 43.3% showed weak focal positivity for Arg-1, compared with all (100%) cases that showed weak focal positivity for GPC3.

Timek *et al.* (2012) noticed a different result in which in the well to moderately differentiated group an equal number of cases stained diffusely strong for both markers, whereas in moderately to poorly differentiated cases Arg-1 was focally strong in 29% and focally weak in 14% of cases, whereas GPC3 demonstrated focal strong staining only in 14% and focal weak staining in 57%, similar to our results.

In the present work, 34 of 74 (46%) HCC cases showed a strong and diffuse staining pattern for Arg-1, compared with 17.1% for GPC3. In contrast, 17.5% of the present cases showed weak focal positivity for Arg-1 compared with 55.7% for GPC3. Thus, Arg-1 allows easier interpretation especially in cytologic samples with small-sized biopsy material having a limited number of cells. In a similar manner, Fujiwara *et al.* (2012) have found that Arg-1 demonstrated diffuse staining pattern in 57% of HCC cases compared with GPC3, where it was found only in 32%.

From the present results we conclude that both markers are sensitive and specific for differentiating HCC from metastatic adenocarcinoma; however, Arg-1 demonstrated better sensitivity and specificity than GPC3. A larger number of cases in higher-grade HCC stained positively for Arg-1 compared with GPC3; 46% of cases showed a strong and diffuse staining pattern for Arg-1 compared with 17.1% for GPC3, and 17.5% showed weak focal positivity for Arg-1 compared with 55.7% for GPC3, which allows better interpretation especially in small FNA biopsy material.

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Conflicts of interest

There are no conflicts of interest.

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