

## The Role of Arginase-1 and Glypican-3 Immune Markers in Differentiating Hepatocellular Carcinoma from Metastatic Carcinoma in Liver Using Cell Block Preparation

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### Abstract

**Background:** Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of liver. Accurate diagnosis and differentiation of HCC by FNAC is often a problem particularly in case of poorly differentiated carcinoma and if the material is insufficient. The cell block method yielded more cellularity and provided better morphological details. Immunohistochemistry (IHC) with the use of Arginase-1 and Glypican-3 immune marker in cell blocks prepared from aspirated material can help to differentiate HCC from metastatic tumor. **Materials and Methods:** This cross-sectional study was carried out in Pathology department of Bangabandhu Sheikh Mujib Medical University from March 2017 to December 2018 with the aim to study the role of Arginase-1 and Glypican-3 IHC markers in differentiating HCC from metastatic carcinoma in liver space-occupying lesion (SOL). **Results:** In this study, mean age was 52.3± 15.1 years. After final diagnosis, 23 (92%) cases were confirmed as HCC, one (4%) as metastatic carcinoma and one (4%) as hepatocellular dysplasia. Arginase-1 was positive in 26 cases. Out of these, 25 cases were finally diagnosed as positive for HCC (true positive) and one case as negative for HCC (false positive). Arginase-1 was negative in 14 cases (true negative). Glypican-3 was positive in 24 HCC cases (true positive). Glypican-3 was negative in 16 cases. Out of these 16 cases, one case was finally diagnosed as positive for HCC (false negative) and 15 cases were finally diagnosed as negative for HCC (true negative). **Conclusion:** Use of cell block and IHC using Arginase-1 and Glypican-3 to differentiate HCC from metastatic tumor will enrich our knowledge and improve patient welfare.

**Keywords:** Liver, Hepatocellular carcinoma, Cell block, Immunohistochemistry.

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### Introduction

Liver cancer was the sixth most commonly diagnosed cancer and the fourth leading cause of cancer death worldwide in 2018, with about 841,000 new cases and 782,000 deaths annually. The highest rate of incidence of liver cancer is found in Mongolia.<sup>1</sup> 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

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of chronic hepatitis C infection and steatohepatitis attributed to the obesity epidemic.<sup>2</sup>

Primary liver cancer includes hepatocellular carcinoma (HCC) (comprising 75%-85% of cases) and intrahepatic cholangiocarcinoma (comprising 10%-15% of cases) as well as other rare types. The main risk factors for HCC are chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), aflatoxin-contaminated foodstuffs, heavy alcohol intake, obesity, smoking, and type 2 diabetes. The major risk factors vary from region to region. In most highrisk HCC areas (China, Eastern Africa), the key determinants are chronic HBV infection and aflatoxin exposure, whereas in other countries (Japan, Egypt), HCV infection is likely the predominant cause. In Mongolia, HBV and HCV virus and coinfections of HBV carriers with HCV or hepatitis D (Delta) virus, as well as alcohol abuse, all contribute to the high burden. The rising obesity prevalence is considered a contributory factor to the observed increasing incidence of HCC in

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low-risk HCC areas.<sup>1</sup>

Bangladesh lies in the intermediate zone regarding the prevalence of HBV. The Prevalence of HCC is 35% among all liver diseases in Bangladesh. The prevalence of HBsAg and HCV positivity are 5.4% and 0.8% respectively in healthy population.<sup>3</sup> The incidence rate of liver cancer in Bangladesh is 2.39% and the number of new cases and deaths annually are 3128 and 2841 respectively. Among all cancers, liver cancer is in the 11th position.<sup>4</sup>

Liver is also a common site for metastatic tumors, accounting for 25% of all metastases to solid organs. Common primary sites from which metastasis occurs are stomach, colon, lung, breast, thyroid and kidney. The comparative morphologic evaluation of HCC and their mimics is often a challenging issue for definitive diagnosis. Some of these diagnostic challenges are: a) the variety of neoplasms that can arise from the hepatic cells; b) metastasis that can mimic variants of primary HCC; c) the diagnostic limitations of serum alpha-fetoprotein (AFP); and d) the distinction of poorly differentiated HCC from cholangiocarcinoma and metastatic carcinoma.<sup>5</sup>

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. Because it is a less-invasive method with relatively low cost, rapid turnaround time and complication rate lower than that of core needle biopsy.<sup>6</sup> It has high sensitivity (100%) but low specificity (63.3%).<sup>7</sup> Although 80% of malignant lesions of the liver can be correctly diagnosed through cytomorphological analysis and good clinico-radiologic correlation, around 20% can pose differential diagnostic problems.<sup>8</sup> This diagnostic pitfall exist in the morphologic distinction of HCC from metastatic carcinoma, particularly in small biopsy material with inadequate sampling and in poorly differentiated tumors. A clear distinction between HCC and metastatic tumors in the liver is clinically important as it carries a significant impact on subse-

quent prognosis and therapeutic management.<sup>9</sup>

Cell block prepared from residue of fine needle aspiration can be useful adjunct to smears for establishing a more definitive cytopathologic diagnosis. It can be particularly useful for categorization of tumor with the help of histochemical stain and immunohistochemistry (IHC) which may not be possible from smears.<sup>10</sup> The sensitivity of cell block varies from 60%- 86% depending on sampling type, type of specimens and aspiration techniques.<sup>11</sup> If conventional smear and cell block are combinedly used, the diagnostic and subtyping accuracy is significantly improved.<sup>12</sup>

The application of adjunct diagnostic tools, such as immunocytochemical staining, which can be selected on the basis of suspected diagnoses, is sometimes essential for a definitive diagnosis.<sup>9</sup> 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 alpha-fetoprotein. However, the utility of each of these markers is limited either by suboptimal sensitivity or by difficulty in interpretation.<sup>13</sup>

Arginase-1 (Arg-1) is a newly described marker in some studies. It is a key urea cycle metalloenzyme and is involved in the hydrolysis of arginine to ornithine and urea. It is recently recognized as a sensitive and specific marker for hepatocytes, both in benign and malignant hepatocytes.<sup>14</sup> In sections of normal liver and HCC, anti Arg-1 produce strong, diffuse cytoplasmic and nuclear 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.<sup>15</sup>

Glypican-3 (GPC3) is a member of the heparan sulfate proteoglycan family. It is an oncofetal protein that is expressed in the embryo and involved in morphogenesis and growth control during development. It produces cytoplasmic,

membranous, and canalicular staining. GPC3 is a specific immunomarker for HCC that can be used to distinguish HCC from benign hepatocellular mass lesions, particularly hepatocellular adenoma.<sup>16</sup> Immunocytochemical staining for GPC3 in cell block material is a highly sensitive and specific method capable of distinguishing HCC from the vast majority of metastatic carcinomas of the liver. Poorly differentiated cases showed the highest GPC3 sensitivity (100%), followed by moderately differentiated cases (96.5%), while well differentiated cases expressed GPC3 in 90% of cases. 83.3% of metastatic carcinomas are negative for GPC3.<sup>8</sup>

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 and also in differentiating benign from malignant hepatic lesion. Arginase-1 demonstrates superior sensitivity compared with GPC-3 and HepPar-1 in the diagnosis of HCC, whereas GPC-3 demonstrates superior specificity, as staining is not observed in benign hepatocellular lesions. Hence, use of Arginase-1 with HepPar-1 and GPC-3 can aid in the diagnosis of HCC and separating from metastatic carcinoma.<sup>2</sup>

## Materials and Methods

**Methodology:** This was a cross-sectional observational study carried out at the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh; from March 2017 to December 2018. Permission for the study was taken from the concerned departments and Institutional Review Board (I.R.B.) of BSMMU, Dhaka. The sample size was 40 and non-random purposive sampling method was followed.

### Inclusion criteria:

- Patients with suspected malignant hepatic mass detected by radiology and imaging and physically fit to sustain FNA procedure.

### Exclusion criteria:

- Cases which are benign both in cytologic smear and cell block.
- Inadequate material.
- Patients having bleeding disorders.
- Non-cooperative patient.
- Cases not accompanied by complete clinical information.

**Selections of patients:** A total of 40 cases having suspected malignant hepatic space-occupying lesion (SOL) with good coagulation profile was selected for USG guided FNAC. Informed written consent was taken from the patients or from their guardians in all cases. According to standard protocol, FNAC was done by an expert physician under local anesthesia in the Hepatology and Radiology department, BSMMU and also in the Radiology department, DMCH.

**Clinical information:** Relevant clinical information was taken. These include- Age and sex of the patients, Relevant history, Clinical features, Laboratory data and Radiological & imaging findings. The data were obtained either directly from the patients or from history sheets in a pre-designed proforma before doing aspiration. All the necessary and relevant data were recorded methodically in a prepared proforma.

**Sample collection:** Under ultrasonography guidance, FNAC was performed by an expert pathologist, radiologist or hepatologist. Cytology smears were made from the aspirated material and immediately fixed in 95% ethanol. Remainder of the aspirate was submitted for cell block and kept in 10% neutral buffered formalin.

**Sample processing:** Several cytological smears were prepared and fixed immediately in 95% alcohol. The smears were left in alcohol for at least 30 minutes at room temperature before staining. Cell blocks prepared from the residual aspirated material were fixed in 10% neutral buffered formalin and later processed to prepare paraffin embedded blocks according to the standard protocol followed at the department of pathology, BSMMU.

**Cytological examination:**

1. **Staining:** Smears prepared from FNA were stained by Papanicolaou and hematoxylin & eosin stain according to the standard staining procedure followed at the department of pathology, BSMMU.
2. **Cytopathological examination:** The stained slides were examined on the next day. Satisfactory smears containing adequate number of representative cells were examined to evaluate the nature of the lesions and presence of malignant cells. Cases were diagnosed according to well established morphologic criteria related to the pattern of cellular arrangement, cellular and nuclear details, and background characteristics.

**Examination of cell block:** Serial sections of paraffin embedded cell blocks were prepared for routine histopathological examination. The sections were cut at 4-6 micro meter thickness and stained with hematoxylin and eosin (H&E) stain by following its' staining protocol. Sections of cell blocks were examined to assess the cellularity and arrangement of the cells. Histopathological assessment of the slides were done according to WHO morphologic criteria.<sup>17</sup>

**Immunohistochemical staining of Arginase-1 and Glypican-3:** Only cases with adequate cellular material on the cell block sections were selected for immunohistochemistry. For each case, one hematoxylin and eosin stained slide from each block was prepared for evaluation of adequacy. Additional two sections were prepared on commercially available Poly-L-Lysine coated glass slides for immunohistochemical stain. Immunohistochemistry was done using the DAKO EnVision TM FLEX, (Low pH Detection system, peroxidase/DAB+, Mouse). In this system primary antibody, anti- Liver Arginase mouse monoclonal antibody (ARG1/1125) and anti-Glypican-3, Cell Marque (clone 1G12) were used. Secondary antibody conjugated to an enzyme (horseradish peroxidase)

labeled polymer (dextran) chain was used subsequently. All slides were counterstained with hematoxylin. Appropriate positive control (histologic sections of known case of HCC for Arg-1 and GPC3) was used. The whole staining procedure was performed in the IHC lab of BSMMU following its standard staining protocol. Immunostained sections were examined by light microscopy under 10x, 20-40x objective lenses.<sup>18</sup>

The staining intensity was graded as strong (clearly seen with x4 or x10 objective lens) or weak (visible at x20 objective or higher) and distribution was recorded as negative (<5% of tumor cells stained), focal (5% -50% staining) and diffuse (>50% staining).<sup>19</sup>

**Analysis of result:** Statistical analysis of the results was obtained by using window-based computer software device with statistical Packages for Social Sciences (SPSS-20). Categorical data were expressed in percentage or number. Parametric data were expressed in mean  $\pm$  SD. The results were calculated by using statistical formulas and presented in tables, figures and diagrams.

**Results**

This cross-sectional study was carried out in Pathology department of Bangabandhu Sheikh Mujib Medical University from March 2017 to December 2018 with the aim of study the role of Arginase-1 and Glypican-3 immuno markers in differentiating hepatocellular carcinoma from metastatic carcinoma in liver SOL. Out of total 40 cases, most of the study patients (45%) were in age between 31-50 years. The mean age was  $52.3 \pm 15.1$  years with age ranging from 26 to 85 years (Figure 01). In this study, 32 (80%) cases were male with a male to female ratio of 4:1 (Figure 02).

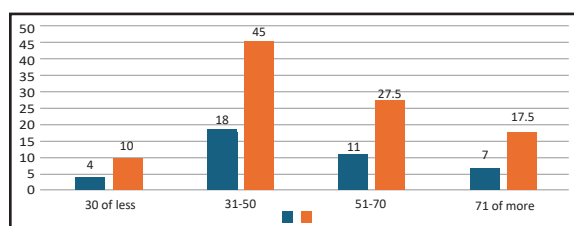


**Figure- 01: Age of the study cases, (n=40)**

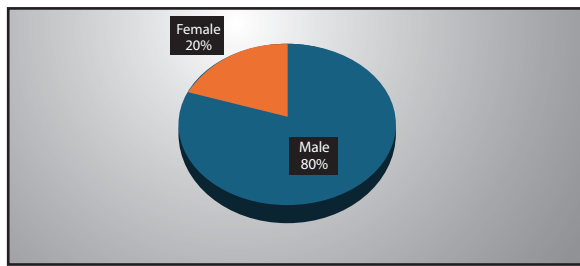


**Figure- 02: Sex of the study cases (n=40)**

The detailed cytomorphology of HCC and metastatic carcinoma and expression pattern of Arginase-1 and Glypican-3 immune markers in cell blocks were also assessed. A total of 40 patients having radiologically suspected malignant hepatic mass and physically fit to sustain the FNAC procedure and fulfilling the other selection criteria was included in this study. Cases which are benign both in cytologic smear and cell block sections, inadequate material, patients having bleeding disorders, and not accompanied by complete clinical information were excluded from this study. Among the 40 study cases, 25 (62.5%) cases were diagnosed as hepatocellular carcinoma, 9 (22.5%) were metastatic carcinoma, 1 (2.5%) was hepatocellular dysplasia and 1 (2.5%) was negative for malignancy. Four (10%) cases were positive but not categorized as HCC or metastatic carcinoma. These were diagnosed as adenocarcinoma either primary or metastatic (Figure 03). After preparation of smear from the fine needle aspirates on the glass slides, the residual material was routinely processed as cell block. On cell block examinations, 26 (65%) cases were diagnosed as hepatocellular carcinoma and remaining 14 (35%) were diagnosed as metastatic carcinoma (Figure 04).

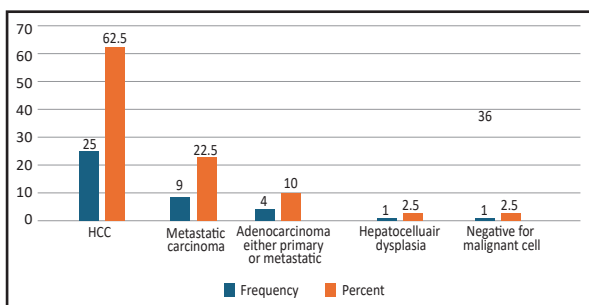


**Figure - 03: FNAC diagnosis of the study patients (n=40)**

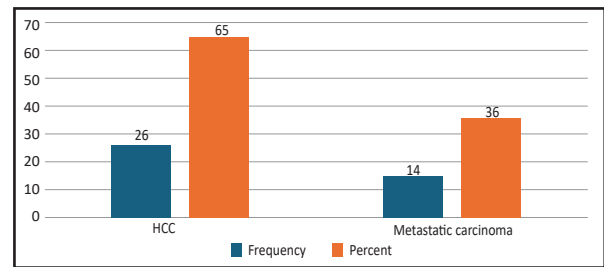


**Figure 04: Cell block diagnosis of the study patients (n=40)**

Subsequently, immunohistochemistry was performed on cell block sections using Arginase-1 and Glypican-3 immuno markers to classify and confirm the diagnosis. Among the total 40 cases, 25 (62.5%) cases were finally diagnosed as hepatocellular carcinoma, 14 (35%) cases as metastatic carcinoma and one (2.5%) case was diagnosed as hepatocellular dysplasia. Arginase-1 immuno stain was positive in all the finally diagnosed 25 (100%) HCC cases and also in case of hepatocellular dysplasia. It was negative in all the 14 (100%) metastatic carcinoma cases. Among the 25 cases of HCC, 24 (96%) was positive for GPC3 and one (4%) was negative. All the 14 (100%) metastatic carcinoma cases and the hepatocellular dysplasia case were negative for GPC3 stain (Table 1) 22 (88%) of the positive cases were diffusely stained with Arg-1 and 17 (68%) were with GPC3. Intensity was strong in 23 (92%) cases for Arg-1 and 17 (68%) cases for GPC3. The staining intensity was graded as strong (clearly seen with x4 or x10 objective lens) or weak (visible at x20 objective or higher). Distribution was recorded as negative (<5% of tumor cells stained), focal (5% -50% staining) and diffuse (>50% staining) (Table 2).

Out of 26 cases positive for HCC by cell block diagnosis, 25 were finally diagnosed as positive for HCC (true positive) and one case as negative for HCC (false positive). Out of 14 negatives for HCC cases in cell block, all were finally diagnosed as negative for HCC (true negative) (Table 3). Arginase-1 was positive in 26 cases. Out of these 26, 25 cases were finally diagnosed as positive for HCC (true positive) and one case as negative for HCC

**Table 1: Interpretation of Arginase-1 and Glypican-3 immuno-stain in HCC, metastatic carcinoma and hepatocellular dysplasia, n=40.**

| Immuno marker     | Hepatocellular carcinoma (%) | Metastatic carcinoma (%) | Hepatocellular dysplasia (%) | Total |
|-------------------|------------------------------|--------------------------|------------------------------|-------|
| <b>Arginase-1</b> |                              |                          |                              |       |
| Positive          | 25 (100)                     | 0 (0)                    | 1 (100)                      | 26    |
| Negative          | 0 (0)                        | 14 (100)                 | 0                            | 14    |
| <b>Glypican-3</b> |                              |                          |                              |       |
| Positive          | 24 (96)                      | 0 (0)                    | 0                            | 24    |
| Negative          | 1 (4)                        | 14 (100)                 | 1 (100)                      | 16    |
| <b>Total</b>      | 25                           | 14                       | 1                            | 40    |

**Table 2: Distribution and intensity of Arg-1 and GPC-3 immunomarker in HCC (n=25)**

|                     |                | Arginase-1 n (%) | Glypican-3 n (%) |
|---------------------|----------------|------------------|------------------|
| <b>Distribution</b> | <b>Focal</b>   | 3 (12)           | 7 (28)           |
|                     | <b>Diffuse</b> | 22 (88)          | 17 (68)          |
| <b>Intensity</b>    | <b>Weak</b>    | 2 (8)            | 7 (28)           |
|                     | <b>Strong</b>  | 23 (92)          | 17 (68)          |
| <b>Total</b>        |                | 25               | 24               |

(false positive). Arginase-1 was negative in 14 cases. All of these were finally diagnosed as negative for HCC (true negative) (Table 4).

**Table 3: Comparison between cell block diagnosis with final diagnosis for HCC; HCC, hepatocellular carcinoma**

| Cell block diagnosis           | Final diagnosis         |                         |
|--------------------------------|-------------------------|-------------------------|
|                                | Positive for HCC (n=25) | Negative for HCC (n=15) |
| <b>Positive for HCC (n=26)</b> | 25<br>(True positive)   | 1<br>(False positive)   |
| <b>Negative for HCC (n=14)</b> | 0<br>(False negative)   | 14<br>(True negative)   |

**Table 4: Comparison between cell block IHC diagnosis by Arginase-1 with final diagnosis, n=40.**

| Cell block IHC diagnosis              | Final diagnosis         |                         |
|---------------------------------------|-------------------------|-------------------------|
|                                       | Positive for HCC (n=25) | Negative for HCC (n=15) |
| <b>Positive for Arginase-1 (n=26)</b> | 25<br>(True positive)   | 1<br>(False positive)   |
| <b>Negative for Arginase-1 (n=14)</b> | 0<br>(False negative)   | 14<br>(True negative)   |

Glypican-3 was positive in 24 cases, all of which were finally diagnosed as positive for HCC (true positive). Glypican-3 was negative in 16 cases. Out of these 16 cases, one case was finally diagnosed as positive for HCC (false negative) and 15 cases were finally diagnosed as negative for HCC (true negative) (Table 5). The validity of Arginase-1 in the diagnosis of HCC was measured by calculating sensitivity (100.0%), specificity (93.3%), accuracy (97.5%), positive predictive value (96.15%) and negative predictive value (100.0%). The validity of Glypican-3 in the diagnosis of HCC was measured by calculating sensitivity (96.0%), specificity (100.0%), accuracy (97.5%), positive predictive value (100.0%) and negative predictive value (93.75%) (Table 6).

**Table 5: Comparison between cell block IHC diagnosis by Glypican-3 with final diagnosis, n=40**

| Cell block IHC diagnosis              | Final diagnosis         |                         |
|---------------------------------------|-------------------------|-------------------------|
|                                       | Positive for HCC (n=25) | Negative for HCC (n=15) |
| <b>Positive for Glypican-3 (n=24)</b> | 24<br>(True positive)   | 0<br>(False positive)   |
| <b>Negative for Glypican-3 (n=16)</b> | 1<br>(False negative)   | 15<br>(True negative)   |

**Table 6: Sensitivity, specificity, accuracy, positive and negative predictive values of Arginase-1 and Glypican-3 immuno marker in differentiation of HCC from metastatic carcinoma.**

| Test of validity                 | Percentage |            |
|----------------------------------|------------|------------|
|                                  | Arginase-1 | Glypican-3 |
| <b>Sensitivity</b>               | 100.0      | 96.0       |
| <b>Specificity</b>               | 93.33      | 100.0      |
| <b>Accuracy</b>                  | 97.5       | 97.5       |
| <b>Positive predictive value</b> | 96.15      | 100.0      |
| <b>Negative predictive value</b> | 100.0      | 93.75      |

## Discussion

This cross-sectional study was carried out in Department of Pathology, Bangabandhu Sheikh Mujib Medical University from March 2017 to December 2018 with an aim to study the role of Arginase-1 and Glypican-3 immuno markers in differentiating hepatocellular carcinoma from metastatic carcinoma in liver using cell block preparation. In this study, the detailed cytomorphology of HCC and metastatic carcinoma and the expression pattern of Arginase-1 and Glypican-3 immune markers in cell blocks were assessed.

A total number of 40 patients having radiologically suspected malignant hepatic mass, physically fit to sustain the FNAC procedure and fulfilling the other selection criteria were included in this study. More than three fourths (80%) of the patients were male and 20% were female. Male to female ratio was 4:1. This indicates that hepatocellular carcinoma is predominant in male patients.

In this study, it was observed that 45% of the patients were in 4<sup>th</sup> decade and above. The mean age was  $52.3 \pm 15.1$  years with age ranging from 26 to 85 years. In case of HCC patients, the majority of patients (40%) belonged to age group 31-50 years and the lowest (12%) patients belonged to age  $\leq$  30 years.

In the current study, according to cytomorphological features, 25 (62.5%) cases were diagnosed as hepatocellular carcinoma, 9 (22.5%) cases as metastatic carcinoma, 1 (2.5%) as hepatocellular dysplasia and 1 (2.5%) case as negative for malignancy. In four (10%) cases the features were overlapping between HCC and metastatic tumor. These indeterminate cases could not be categorized. These were diagnosed as adenocarcinoma either primary or metastatic. Few authors showed in their study, 70% of lesions were diagnosed as HCC, representing the majority of the cases and 30% cases were diagnosed as metastatic carcinoma.<sup>8</sup> Similarly, some authors reported that 58.5% of

malignant hepatic tumors were HCC and 41.5% were metastatic origin. In the current study, the cell block diagnosis was HCC in 26 (65%) cases and metastatic carcinoma in 14 (35%) cases.<sup>20</sup>

The final diagnosis was made on the basis of cytomorphology of neoplastic cells, clinico-radiological correlation, serum tumour markers, cell block assessment and immunohistochemistry with Arginase-1 and Glypican-3 immuno marker on cell block sections. Same diagnostic criteria were also followed by a group of authors.<sup>8</sup> According to the final diagnosis, the distribution of the patients in this current study was HCC in 25 (62.5%) cases, metastatic carcinoma in 14 (35%) cases and hepatocellular dysplasia in one (2.5%) case.

In this study, Arginase-1 immuno stain was positive in all the 25(100%) HCC cases and also in a single case of hepatocellular dysplasia. It was negative in all the 14 (100%) cases of metastatic carcinoma. Tahoun & Ezzat, (2013) showed that 96.1% cases of HCC were positive for Arginase-1. Among the 25 cases of HCC, 24 (96%) cases were positive for Glypican-3 and one (4%) case was negative. All the metastatic carcinoma 14 (100%) cases and hepatocellular dysplasia case were negative for Glypican-3 immunostain.<sup>9</sup> Zaakook et al. (2013) showed in their study that Glypican-3 was positive in 95.2% of HCC.<sup>8</sup>

In this study, the arginase-1 immunostain was positive in 25 cases. The staining was cytoplasmic or cytoplasmic and nuclear in hepatocytes. Three (12%) cases were focally and 22 (88%) were diffusely positive. The intensity was weak in two (8%) and strong in 23 (92%) cases. No Arginase-1 staining was found in 14 cases. Glypican-3 immunostain was positive in 24 cases. The staining was cytoplasmic and membranous in neoplastic hepatocytes. Seven (28%) of these were focally and 17 (68%) were diffusely positive. The intensity was weak in seven (28%) cases and strong in 17 (68%) cases. No Glypican-3 staining was seen in 16 cases.

In the present study, out of total 40 cases, cytological diagnosis was HCC in 25 patients. After final diagnosis, out of these 25 HCC cases, 23 (92%) cases were confirmed as HCC, one (4%) as metastatic carcinoma and one (4%) as hepatocellular dysplasia. Nine patients were cytologically diagnosed as metastatic carcinoma, out of which one (11.1%) case was confirmed as HCC and remaining eight (88.9%) cases were confirmed as metastatic carcinoma. Single case of hepatocellular dysplasia was finally confirmed as HCC. Single case which was negative for malignancy in cytology was confirmed as metastatic carcinoma. Four cases cytologically were positive but not conclusive and diagnosed as adenocarcinoma either primary or metastatic. These four cases were finally diagnosed as metastatic carcinoma.

The study showed that cytologically diagnosed hepatocellular carcinoma were true positive in 23 (92%) cases, false positive in 2 (8%) cases, false negative in 2 (8%) cases and true negative in 13 (52%) cases according to final diagnosis. In this study cytologically diagnosed metastatic carcinoma were true positive in eight (57.1%) cases, false positive in one (7.14%) case, false negative in six (42.85%) cases and true negative in 25 (96.15%) cases according to final diagnosis. So, the overall sensitivity and specificity of cytological diagnosis was 92.0 % and 86.67% respectively for the diagnosis of HCC. And for the diagnosis of metastatic carcinoma, the sensitivity and specificity were 57.1% and 96.1% respectively. Various studies have reported sensitivity varying from 67% to 99.5% and specificity 87% to 100%. Swamy et al. 2011 and Zaakook et al. (2013) found that 80% of HCC can be correctly diagnosed by cytomorphological analysis and good clinico-radiological correlation.<sup>8,21</sup>

In this study, Arginase-1 was positive in 26 cases. Out of these 26 cases, 25 cases were finally diagnosed as positive for HCC (true positive) and one case as negative for HCC (false positive). Argin-

ase-1 was negative in 14 cases. Out of these 14 cases, all were finally diagnosed as negative for HCC (true negative). So, regarding the validity of Arginase-1 immunostaining in differentiating HCC from metastatic carcinoma with sensitivity, specificity, positive predictive value, negative predictive value and total accuracy were 100%, 93.33%, 96.15%, 100% and 97.5% respectively. Few study showed, Arginase-1 achieved a specificity ranging from 87 to 100%.<sup>2, 14, 22, 23</sup> The specificity for Arginase-1 detected in the current work (93.33%) agreed with the values observed in previous studies. In the present study, Glypican-3 was positive in 24 cases, all of which were finally diagnosed as positive for HCC (true positive). Out of 16 negative Glypican-3 cases, one case was finally diagnosed as positive for HCC (false negative) and 15 cases were finally diagnosed as negative for HCC (true negative). So, regarding the validity of Glypican-3 immunostaining in differentiating HCC from metastatic carcinoma with sensitivity, specificity, positive predictive value, negative predictive value and total accuracy were 96.0%, 100.0%, 100.15%, 93.75% and 97.5% respectively. Other studies showed sensitivity ranging from 72% to 95% and specificity between 96% to 100%.<sup>8,16,20</sup> The current study showed that both Arginase-1 and glypican-3 have higher sensitivity and specificity in differentiating hepatocellular carcinoma from metastatic carcinoma.

### **Limitations of the study:**

Sample size was small. Study population was selected from tertiary care hospital, so it may not reflect the exact picture of the country. Follow up biopsy was not available.

### **Conclusion**

Both Hepatocellular and metastatic carcinoma are common in liver. A clear distinction between HCC and metastatic carcinoma is important because it carries a significant impact on subsequent prognosis and therapeutic management. The distinction between these tumors is often difficult by

cytology alone. The current study showed that, cell block prepared from aspirated material and stained with Arginase-1 and Glypican-3 immunomarkers has high sensitivity and specificity in differentiation of HCC from metastatic carcinoma. So, it can be concluded that cell block with immunohistochemistry using Arginase-1 and Glypican-3 immuno-panel in conjunction to cytology is a very useful diagnostic modality in differentiating HCC from metastatic carcinoma in suspicious cases.

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