



Original Article

Diagnostic Utility of Cell Block and Glypican-3 Immunostaining in Differentiating Hepatocellular Carcinoma from Hepatic Metastases at a Rural Tertiary Care Center

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Received: 10-05-2026

Accepted: 16-07-2026

Available online: 17-07-2026

ABSTRACT

Introduction: World-wide hepatocellular carcinoma (HCC) is the sixth most common cancer and fourth leading cause of cancer related deaths. Metastases are about 18- 40 times commoner than HCC and have some morphological similarities with it, but their prognosis and treatment approaches vary. Fine needle aspiration cytology (FNAC) alone faces some diagnostic challenge to differentiate between poorly differentiated HCC and metastatic carcinoma. Cell block preparation utilizing material obtained through ultrasound (USG) guided FNAC of liver tumors is easy and is a safer procedure than more invasive core needle biopsy. Glypican-3 (GPC-3), a member of heparan sulfate proteoglycan family, when used on histopathology sections, has an important diagnostic role in differentiating HCC from other hepatic mimickers.

Objective: The present study aimed at assessing the diagnostic utility of Glypican-3 IHC stain on cell block preparation for differentiating primary from secondary hepatic malignancies. No such study has thus far been conducted from our sub-himalayan region.

Materials And Methods: The present study was an institution based observational study with cross sectional design conducted in a tertiary care centre of Eastern India among patients referred to Department of Pathology for USG guided FNAC of hepatic mass and suspected to have HCC or metastases clinically and / or radiologically. Under USG guidance, FNAC was performed twice followed by core needle biopsy from the hepatic mass. Cell blocks were prepared from the second pass aspirate by plasma-thrombin clot method. The cell block and core needle biopsy specimens were stained by hematoxylin and eosin (H&E) and subjected to immunohistochemistry (IHC) using Glypican-3 IHC by labelled Horse Radish Peroxidase (HRP) polymer technique. The diagnostic utility of Glypican-3 IHC staining was analyzed with the help of core needle biopsy results as a gold standard.

Results: The study population comprised of 60 cases of hepatic SOL, among which on core needle biopsy, 24 were diagnosed as HCC and 36 as metastatic carcinoma in liver. The sensitivity, specificity and diagnostic accuracy of FNAC was found to be 87.5%, 83.4% and 85% respectively. The same for cell block preparation came out to be 91.7%, 88.9% and 90% respectively. When Glypican-3 IHC stain was done on cell block preparation, the sensitivity, specificity and diagnostic accuracy increased to 95.8%, 94.5% and 95% respectively.

Conclusion: The present study revealed that in cases of HCC, diagnostic accuracy of cell block is greater than FNAC in differentiating it from hepatic secondaries. IHC staining for Glypican-3 in cell block preparation is an even more sensitive and specific and is a valuable tool capable of differentiating HCC from metastatic carcinoma of liver.

INTRODUCTION

Hepatocellular Carcinoma (HCC) is the sixth most common cancer and fourth leading cause of cancer related deaths worldwide [1,2,3]. Metastases are the commonest malignant liver lesions [4] and are about 18-40 times more common than primaries [5]. HCC may have some morphological similarities with metastases but their prognosis and treatment approaches vary [6]. The diagnosis of HCC and differentiating it from metastases is based on clinical background, imaging findings, serum alpha-fetoprotein (AFP) and pathologic analysis [7]. However the sensitivity and specificity of Ultrasonography (USG) and AFP are not satisfactory enough to detect HCC [8]. Ultrasound/ computerized tomography (USG/ CT) guided Fine Needle Aspiration Cytology (FNAC) is presently a rapid, low cost, safe and accurate procedure to establish a diagnosis of focal hepatic mass lesion as either primary or metastatic. [1,9,10,11,12] Multiple passes from any region of liver are easy to obtain, allowing for better sampling by FNAC [10]. However, FNAC alone faces some diagnostic challenges to differentiate between poorly differentiated HCC and metastatic carcinoma [13]. Cell block preparation, utilizing material obtained through USG guided FNAC of liver tumors is easy and is a safer procedure than the more invasive core needle biopsy (CNB) [14,15,16]. As an adjunct to histopathology, immunohistochemistry (IHC) plays a very crucial role in the differential diagnosis of liver tumors [17,18]. The currently available IHC markers used for this purpose, such as alpha-fetoprotein (AFP), HepPar-1, polyclonal carcino-embryogenic antigen (p CEA), CD 10, & CD 34 have significant diagnostic limitations [19]. Glypican-3 is a 60 kDa cell-surface protein, member of heparan sulfate proteoglycan family, which is linked to the cell surface through a glycosyl phosphatidylinositol anchor. It is an oncofetal protein, expressed in the embryo and is involved in morphogenesis and growth control during development, minimally expressed in normal adult tissues. When used on histopathology sections, it has an important diagnostic role in differentiating HCC from other hepatic mimickers [20]. It has a reported sensitivity ranging from 72% to 90% [21-24] and a specificity between 96% and 100% [23]. It has also been suggested that poorly differentiated HCC are more likely to express Glypican-3 [25,26]. So the present study aimed at assessing the diagnostic utility of Glypican-3 IHC stain on cell block preparation in differentiating primary from secondary hepatic malignancies by comparing it with CNB results taken as a gold standard. Even after a diligent search of published literature, no similar study was found from our sub-himalayan region.

Specific Objectives-

- To study the cytological features of primary HCC and metastatic deposits in the liver.
- To observe the immunohistochemical staining pattern of Glypican-3 on cell block preparation in both primary and secondary hepatic malignancies.
- To assess the diagnostic utility of Glypican-3 IHC stain on cell block preparation in differentiating primary from secondary hepatic malignancies.

Methods:

The present study was an institution based observational study with cross sectional design conducted in the Departments of Pathology, Radiology and Surgery at a tertiary care centre of Eastern India from July 2022 to June 2024. Study population consisted of sixty patients (non-probability consecutive sampling) suspected to have hepatocellular carcinoma or metastatic malignancy clinically and/or radiologically and subsequently referred to Department of Pathology for USG guided FNAC of the hepatic mass. Patients with prothrombin time (PT) within normal range with or without Vitamin K therapy were included in the study. All patients unwilling to give proper informed consent or having lesions other than hepatocellular carcinoma or metastatic malignancy as per cytomorphological features were excluded from the study. The study was performed after obtaining prior approval from the institutional ethics committee.

The patients were informed about the study in details and written consent was taken. All relevant clinical and radiological data were collected from the patients and the study proforma was filled.

Materials was aspirated from the liver mass under USG guidance with cameco syringe pistol fitted with 10cc syringe and spinocaine needle (21-23G).

Air dried and alcohol fixed smears were prepared from the materials collected from the 1st pass.

Materials collected from the 2nd pass were preserved in 10% neutral buffered formalin for cell block preparation. After that CNB was performed using BARD Max needle core biopsy gun (22mm).

FNAC smears from 1st pass were stained by Leishman stain (air dried smears) and H&E / PAP stain (alcohol fixed smears).

Cell blocks were prepared from the 2nd pass by plasma-thrombin clot method. After proper processing, paraffin section were stained by H&E stain. CNB specimens were also processed & paraffin sections were stained by H&E stain.

Now the stained cytological smears and the slides prepared from paraffin section of cell block and CNB after proper tissue processing procedure, were ready for interpretation.

Then with the help of the above smears and slides, the cytological diagnosis was made by 3 blinded faculties of Department of Pathology of our college. The diagnosis of cell block and CNB specimen were also made in the same way.

After that immunohistochemical staining was done by labelled Horse Radish Peroxidase (HRP) polymer technique using cell block section with the help of Glypican-3, and the findings was noted (cytoplasmic or membranous staining pattern).

Finally the diagnostic utility of Glypican-3 immunohistochemical staining was analysed with the help of help of CNB results as a gold standard.

Tables / Figures

Fig.-1: PD HCC, H&E stain, 40x

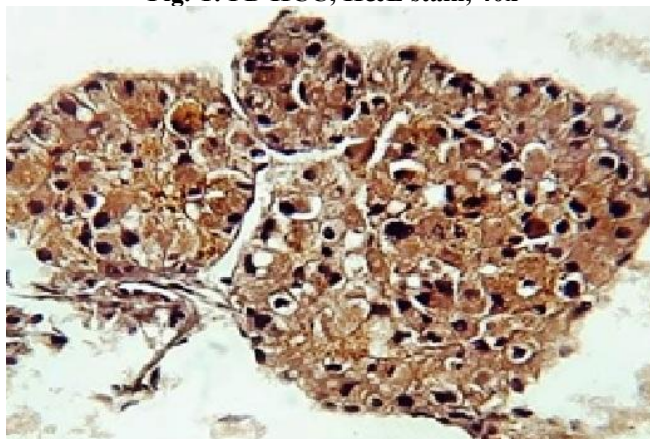


Fig.-2: Metastatic Adenocarcinoma Liver, Leishman Stain, 100x

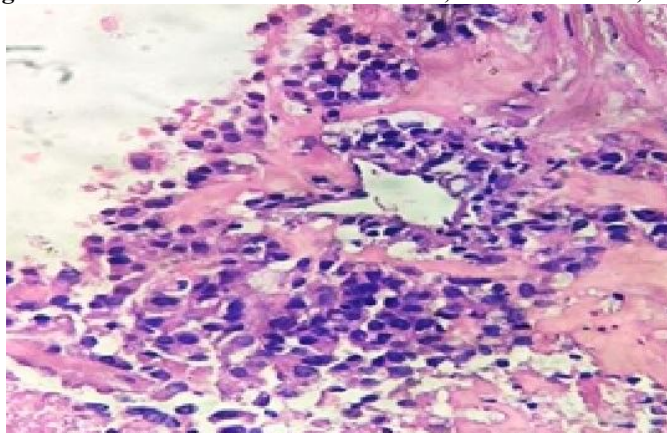


Fig.-3: PD HCC, Cell block, H&Estain, 40x

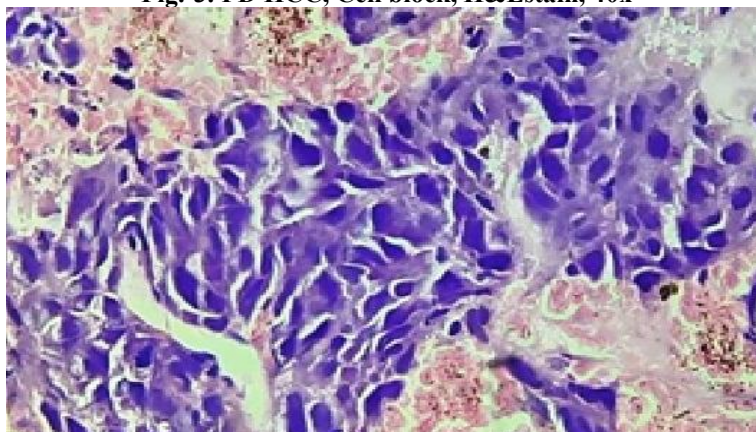


Fig.- 4: Metastatic adenocarcinoma,Cell block, H&E stain, 40x

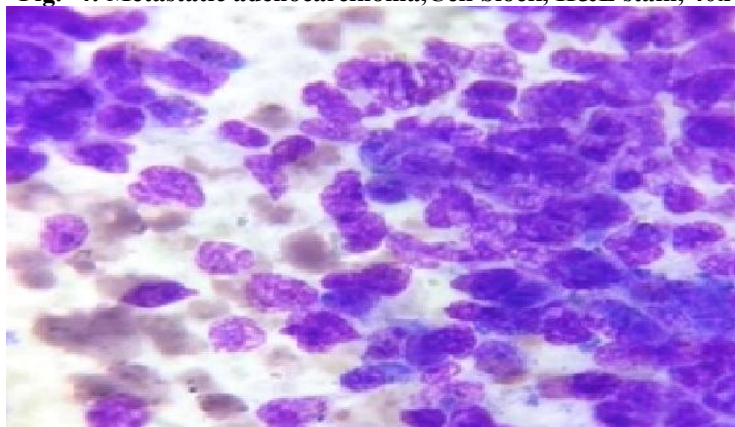


Fig.-5: HCC, Membranous + Cytoplasmic pattern, (GLYPICAN-3), Cell block, 40x

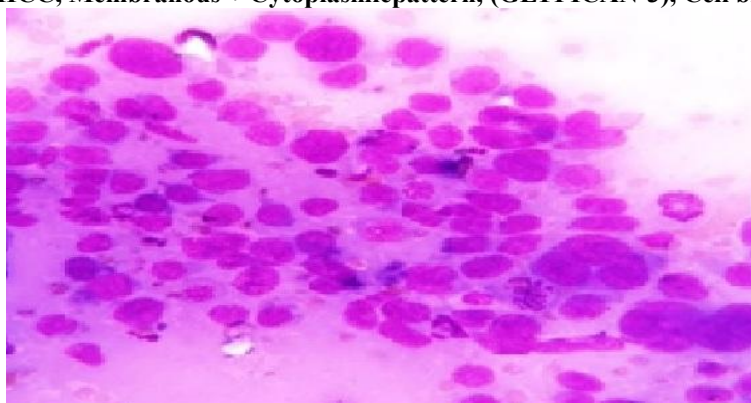


Fig.-6: Metastatic adenocarcinoma,Negative (GLYPICAN-3), Cell block, 40x

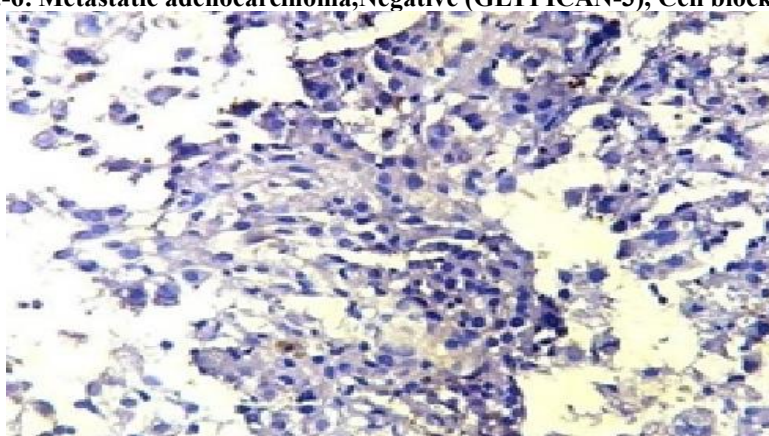


Table-1: Results of FNAC, Cell block, Cell block with IHC (Glypican-3), Core needle biopsy, & CNB with IHC (Glypican-3)

Diagnosis	FNAC	Cell block	Cell block with IHC (Glypican-3)	CNB	CNB with IHC (Glypican3)
HCC	27	26	25	24	24
Metastatic Carcinoma	33	34	35	36	36
Total	60	60	60	60	60

Table-2: Comparison of diagnostic utility of various procedures

Tests	True +ve	True -ve	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic accuracy
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FNAC	21	30	87.50%	83.33%	77.80%	90.90%	85%
Cell block	22	32	91.70%	88.90%	84.61%	94.11%	90%
Cell block with Glypican-3	23	34	95.83%	94.44%	92%	97.14%	95%

On FNAC, HCC shows cellular smears composed of neoplastic cells in trabecular & acinar pattern and also in multilayered sheets. Individual cells are polygonal shaped with increased N: C ratio, have central round nuclei often with macronucleoli. Increased nuclear size and atypia are more in moderately differentiated and poorly differentiated HCC. Bi-nucleation, multinucleation, intranuclear cytoplasmic inclusions and intracytoplasmic bile pigments are present in HCC along with atypical stripped nuclei and frequent mitoses. Hyaline bodies might be present. Bile duct epithelium is usually absent. Cytoplasmic vacuoles might be present.

Metastases show the cytological features of primary tumor itself along with reactive hepatocytes. However poorly differentiated HCC frequently pose a diagnostic dilemma in cytomorphological differentiation between HCC and metastases [10,11,12].

Diagnostic criteria of HCC on cell block [13]

Architectural details such as trabecular-sinusoidal pattern formed by broad trabeculae (>2 cells thick), pseudoacini and unpaired arteries aid HCC diagnosis.

The diagnosis of HCC is based on cyto-architectural features, such as cell atypia, cell crowding, trabecular thickness, and microacini. Establishment of trabeculae ≥ 3 cells thick is one of the most helpful features in diagnosis of highly well-differentiated HCC.

Data Analysis

The collected data was tabulated in Microsoft Excel data sheet. Continuous quantitative variables were expressed as the mean / SD and categorical qualitative variables expressed as absolute frequencies (number) & relative frequencies (percentage). They were analysed using Chi square test and the continuous data using unpaired Student's t test. The results were considered statistically significant when the significant probability was less than 0.05 ($p < 0.05$).

Statistical software used: GraphPad Prism 10 (San Diego, USA), Statistical Package for Social Sciences (SPSS version 24, IBM, USA).

RESULTS:

The study population comprised of 60 cases of hepatic SOL, among which on CNB, 24 were diagnosed as HCC and 36 as metastatic carcinoma in liver. In cases of HCC, age of patients ranged from 50 - 79 years with mean age of 65.25 ± 4.0 (S.D.) years and majority of the patients (83%) were in the sixth decade of life. In patients with metastatic carcinoma, age ranged from 40-89 years with a mean age of 60.86 ± 8.57 (S.D.) years and majority of the patients (56%) were in their fifth decade.

HCC were more common in males (75%) than females (25%) and metastatic carcinoma were also more common in male (66.67%) than female (33.33%). ($p = 0.4902$)

In case of HCC, patients had a clinical history of pain abdomen (100%) followed by weight loss (54%), ascites (29%), jaundice (25%) and anorexia (25%) respectively. There was no history of hematochezia and altered bowel habit. In case of metastatic carcinoma, patient had a clinical history of pain abdomen (97%) followed by jaundice (53%), weight loss (22%), hematochezia (17%), ascites (14%) and altered bowel habit (14%) respectively.

21/24 (87.5%) cases of HCC showed elevated serum AFP level. Non elevation (≤ 20 ng/ml) was seen in 12.5% (3/24) cases. HCC showed mildly elevated (>20 -200 ng/ml), moderately elevated (>200 -400 ng/ml) and markedly elevated (>400 ng/ml) serum AFP level in 16.67% (4/24) cases, 20.83% (5/24) cases, and 50% (12/24) cases respectively. Metastatic carcinoma showed mildly elevated and moderately elevated serum AFP level in 36% (13/36) cases & 14% (5/36) cases respectively. No elevation (≤ 20 ng/ml) cases were seen in 47% (17/36) cases. Marked elevation was seen in a single patient (3%). So serum AFP levels were very significantly higher in HCC. ($p = 0.00005$)

Out of 23 solitary SOL, 19 cases were HCC, and 4 cases were metastatic carcinoma and out of 37 multifocal SOL, 32 were metastatic carcinoma and 5 were HCC. So, HCC showed 19 cases (79%) as a solitary SOL & 5 cases (21%) showed

multifocal masses and the metastatic carcinoma group showed multifocal SOL in 32/36 (89%) cases, and single SOL in 4/36 (11%) cases. Solitary SOLs were seen to be significantly more associated with HCC. ($p < 0.0001$)

HCC was diagnosed in 27, 26, 25, 24 and 24 cases on FNAC (Figure 1 and 2), cell block (Figure 3 and 4), cell block with IHC (Glypican-3) (Figure 5 and 6), CNB and CNB with Glypican-3 IHC stain respectively whereas metastatic carcinoma was diagnosed in 33, 34, 35, 36 and 36 cases respectively (Table1). The sensitivity, specificity and diagnostic accuracy of FNAC was found to be 87.5%, 83.4% and 85% respectively. The same for cell block preparation came out to be 91.7%, 88.9% and 90% respectively. When Glypican-3 IHC stain was done on cell block preparation, the sensitivity, specificity and diagnostic accuracy increased to 95.8%, 94.5% and 95% respectively (Table2).

In relation to cell block out of total 60 cases, 25 cases showed immunoreactivity for Glypican-3 (considered as positive), 35 cases showed no immunoreaction for Glypican-3 (considered as negative). The immunoreactive cases were diagnosed as HCC whereas non-immunoreactive ones were diagnosed as metastases in cell block preparation. Among these 25 Glypican-3 positive cases on cell block, 23 cases (92%) were finally diagnosed as HCC on CNB but 2 (8%) cases provisionally diagnosed as HCC on cell block with Glypican-3 later turned out to be metastatic deposits of ovarian carcinoma in liver and squamous cell carcinoma lung with liver metastasis that was revealed with the help of further investigations and CNB sections. Out of 35 cell block Glypican-3 negative cases, 1 case (2.86%) was finally diagnosed on CNB as moderately differentiated HCC and other 34 metastatic cases (97.14%) turned out to be metastatic deposits in liver in CNB as well. So finally 24 cases of HCC (23 cell block Glypican-3 positive & 1 cell block Glypican-3 negative) and 36 cases of metastatic disease in liver (34 cell block Glypican-3 negative & 2 cell block Glypican-3 positive) were diagnosed based on gold standard CNB with Glypican-3 IHC.

Coming to Glypican-3 staining pattern in HCC, 12 (48%) cases showed membranous pattern, 4 (16%) showed cytoplasmic and 9 (36%) cases showed mixed (membranous with cytoplasmic and membranous with canalicular) pattern. On histopathology, out of 23 Glypican-3 positive cases of HCC, 7 cases (29%) were Well Differentiated HCC, 11 cases (46%) were Moderately Differentiated HCC, & 6 (25%) were Poorly Differentiated HCC along with 1 Glypican-3 negative moderately differentiated HCC.

DISCUSSION:

Majority of HCC patients ranged from 50-79 years which is similar to studies worldwide [27] and in India [28]. Sex distribution of HCC was close to 2:1 to 4:1 Male:Female ratio reported worldwide [29,30]. In an Indian study by Lokesh K N et al. 72.9% patients were male with male : female ratio of 2.7:1 [31]. In cases of metastatic carcinoma, most were aged 40-89 years and majority (56%) were in fifth decade with a male : female ratio of 2:1. Griscom J T et al reported that metastatic carcinoma of liver was more common in males, and majority of the patients were more than 50 years of age [32]. Ridder J D et al also found that metastases more common in males [33]. Mohamed Z et al documented male predominance both in HCC and metastases. [34].

In this study, metastases (36 cases, 60%) was commoner than HCC (24 cases, 40%). Similar results were reported by Chowdhury S et al [35] in West Bengal (2015) and in a study in China (2018) by Chen D et al [25]. But Hemalatha A L et al [36] found 44.4% cases of HCC as compared to metastatic hepatic lesion in 27.7% and Ibrahim T R et al [37] found 62.5% cases of HCC, and 37.5% cases of metastatic carcinoma. Equal number of cases of HCC and metastatic lesion was observed by Ahuja A et al [1].

Majority (83%) of HCCs presented as solitary SOL, and few (17%) showed multifocal masses on USG. The metastatic carcinoma group showed multifocal SOL in 32/36 (89%) cases, and single SOL in 4/36 (11%) cases. Chowdhury S et al [35] and Ahuja A et al [38], have found similar results in their studies.

Reddy C V et al [39] reported FNAC sensitivity, specificity and diagnostic accuracy as 93%, 90.9% and 92.8%, respectively, which corroborated with those observed in the present study. Rastogi N et al [40] and Swamy M C M et al [41] also reported similar findings.

In the present study, on cell block, 26 (43%) cases were diagnosed as HCC and 34 (57%) cases as metastatic carcinoma. In 54 cases (22 HCC and 32 metastases), diagnosis on both cell block and CNB were similar, but on cell block, 4 false positive and 2 false negative cases were found. Haq S et al, [6] reported in their study, that use of cell block helped in reaching a final diagnosis in an additional 29.1% cases, compared to conventional smear. A recent study by Nagose V B et al [42] reported sensitivity (95.77%) and diagnostic accuracy (94.87%) of cell block to be considerably higher than FNAC (78.84% and 78.46% respectively) and they concluded in their study, that cases of suspicious diagnosis by FNAC or diagnosis discordant with clinical findings, should be followed up with cell block to improve the diagnostic yield & to facilitate accurate diagnosis.

Cell block when combined with IHC, in the present study, was able to diagnose 57 cases out of 60 cases (23 HCC, 34 metastatic deposits) accurately with 2 false positive and 1 false negative results. Similar results were reported by Ibrahim

TR et al. [37] Shan-yu Qin et al [43] showed 90% sensitivity, 100% specificity, and 91.7% diagnostic accuracy of cell block with IHC in their study of pancreatic lesions. In the present study, the false negative case was diagnosed as moderately differentiated, nuclear grade II HCC. It corroborated well with findings of Mohamed Z et al [34] who reported that grade III (poorly differentiated) HCC cases showed the highest Glypican-3 expression (100 %), followed by grade II (moderately differentiated) cases (96.5 %), while grade I (well differentiated) cases expressed Glypican-3 in 90 % of cases. The two false positive cases on further investigation were diagnosed as metastatic deposits of ovarian clear cell adenocarcinoma and poorly differentiated squamous cell carcinoma of lung. Both the tumors have been reported to be Glypican-3 immunoreactive in published literature. [46, 47]

Several recent studies have concluded that Glypican-3, when used as a single IHC marker, was much helpful in differentiating HCC from benign hepatic lesions [21,22,23,24,45,48] and from metastatic neoplasms of the liver [49]. It has also been suggested that poorly differentiated HCCs are more likely to express Glypican-3 [23].

Majeed S et al [50] reported Glypican-3 staining was positive in 48.33% cases and negative in 51.67% cases with sensitivity of 82%, specificity 94.33%, positive predictive value (PPV) 94.82% and negative predictive value (NPV) of 80.64%. Diagnostic accuracy was 87.5%. Mohamed Z et al [34] showed Glypican-3 was expressed in the majority of cases of HCC with a sensitivity (95.2%), specificity (83.3%), PPV (93%), NPV (88.2%) and total accuracy of 91.7%. Ibrahim T R et al [37], Geramizadeh et al [18] and Saverio L et al [49] reported similar results.

Immunostaining patterns observed in the present study corroborated well with findings reported in published literature. Wang X Y et al [24], Chen I P et al [51], Wang H L et al [52] and Geramizadeh et al [18] documented 3 different immunostaining patterns of GPC3-expression in HCC: predominantly membranous, predominantly canalicular, and predominantly cytoplasmic. These different staining patterns did not appear to correlate with the differentiation status or the growth pattern of the tumors. In fact, more than one third of the tumors exhibited a mixed staining pattern within the same tumors, although the tumor cells with different staining patterns showed similar or identical histologic features.

Limitation: It was a single institution based study, assessing role of only one IHC marker. Moreover, in a minor percentage of metastatic carcinoma cases, Glypican-3 staining was falsely positive. Use of other HCC specific IHC markers like HepPar1 or MOC31 on cell block preparation may be assessed. Multicentric study with more number of cases, assessing other IHC markers would help in comparing the diagnostic utility of various potential biomarkers.

CONCLUSION:

The present study revealed that in cases of HCC, diagnostic accuracy of cell block is greater than FNAC in differentiating it from metastases. Furthermore, the additional use of Glypican-3 IHC on cell block sections increases the diagnostic accuracy. Immunohistochemical staining for Glypican-3 in cell block preparation of liver is highly sensitive and specific and is a valuable tool capable of differentiating HCC from metastases. More importantly, biopsy can be avoided, which has definitive advantages especially in resource deprived set ups.

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