Original Article

Fine-needle aspiration cytology and biopsy in hepatic masses: A minimally invasive diagnostic approach

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ABSTRACT

Aims and Objective: To evaluate the diagnostic sensitivity, usefulness and limitations of fine-needle aspiration cytology (FNAC) and fine-needle aspiration biopsy (FNAB) in the diagnosis of hepatic masses. **Materials and Methods:** FNAC was performed on 150 cases of hepatic masses under guidance of ultrasound or computed tomography (CT) scan. Adequate diagnostic aspirates were obtained in 147 cases (98.0%). Smears were stained with hematoxylin and eosin (H and E), and Papanicolaou stains. FNAB was obtained from the same 149 cases (except one) and stained with HE stain. The hepatic masses were categorized into benign, malignant and inflammatory groups. **Results:** Out of 150 hepatic masses, 3.3% were benign, 94.26% were malignant and 2% were inflammatory lesions. FNAC and FNAB were unsatisfactory for evaluation in 3 out of the 150 cases (2%) and 6 out of 149 cases (4.02%), respectively. Correct cytological diagnoses were achieved in 129 out of the 150 cases (diagnostic sensitivity: 86%). FNAB gave satisfactory results in 143 out of 149 cases (diagnostic sensitivity: 95.77%). Cytological diagnoses of 21 cases were not consistent with histology (false negativity: 14%). Cyto-histological correlation showed 87.32% diagnostic sensitivity of FNAC for malignant tumors, whereas benign tumors posed maximum diagnostic problems, with sensitivity of 40%. This difference was statistically significant ($P < 0.05$). FNAB showed a statistically significant difference ($P < 0.05$) compared with FNAC in the diagnosis of benign and malignant hepatic masses. FNAC showed 100% diagnostic sensitivity for inflammatory lesions. **Conclusion:** Malignant tumors of liver can be confidently diagnosed on FNAC. However, FNAC has a few limitations and diagnostic challenges in benign lesions, well-differentiated and poorly differentiated hepatocellular carcinoma, and metastatic carcinoma. Microhistology by FNAB allows architectural, cellular and immunohistochemical evaluation. To obtain maximum diagnostic information with reduction of indeterminate reports, a combined approach of FNAC and FNAB with clinical findings, tumor markers and ancillary techniques should be used.

Key words: Benign, fine-needle aspiration biopsy, fine-needle aspiration cytology, hepatocellular carcinoma, liver, malignant, metastatic carcinoma

INTRODUCTION

Accurate diagnoses of hepatic masses are very important because treatment ranges from supportive care for advanced metastatic lesions to partial hepatectomy for primary carcinoma. Radiological imaging and serological markers can be useful in narrowing the differential diagnosis. However, tissue diagnosis is often required to guide subsequent management.[1-4] Fine-needle aspiration cytology (FNAC) is useful in the diagnosis of benign, malignant and inflammatory hepatic lesions under guidance of ultrasound or computed tomography (CT) scan, with low risk of complications.[1-7] Major cytological diagnostic issues arise in benign hepatocellular lesions, reactive hepatocytes, well-differentiated hepatocellular carcinoma (WD-HCC), poorly differentiated HCC (PD-HCC), cholangiocarcinoma, metastatic carcinomas and determination of primary site of metastatic tumor. These lead to indeterminate reports on FNAC.[3,7-10] The advantage of cytodiagnosis is obvious as it may obviate the need for diagnostic laparotomy, especially in inoperable cases, and allows specific chemotherapy to be

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instituted without delay.[3] Microhistology by fine-needle aspiration biopsy (FNAB) provides detailed architecture and allows special stains, including immunohistochemistry application.[3,6] This study evaluates the importance of FNAC and FNAB in the diagnosis of focal hepatic lesions from a pathologist's and hepatologist's perspective and addresses the diagnostic sensitivity, usefulness, limitations and pitfalls of FNAC in the diagnosis of commonly encountered hepatic masses.

MATERIALS AND METHODS

A total 150 cases of hepatic masses were detected clinically and radiologically, and subjected to FNAC and FNAB (except one) during March 2007 to March 2011 prospectively. Clinical, serological and radiological details were obtained from patient and case files. Bleeding time, clotting time and prothrombin time were evaluated in all cases.

The procedure was performed using a 20/21-gauge disposable spinal needle, attached to a 10-ml disposable syringe. The cutting mechanism provided material for cytology and microhistology. Under antiseptic precautions, during suspended respiration, the needle was introduced percutaneously into the lesion under ultrasound or CT scan guidance. When adequate material appeared in the hub, the needle was withdrawn after releasing the suction pressure. One to three passes were done. Monitoring of pulse, respiration and blood pressure was done for 4-6 hours. If no change was found, the patient was discharged. Usually five to seven smears were prepared and fixed in 95% methanol for Papanicolaou (PAP) and hematoxylin and eosin (H and E). FNAB samples from 149 cases were fixed in 10% formalin, processed and embedded in paraffin blocks. Sections were stained by H and E stain. Immunohistochemical stains were done where required. The results of FNAC and FNAB were evaluated and categorized into benign, malignant and inflammatory groups. The diagnostic sensitivity of FNAC was calculated by considering microhistology by FNAB as the gold standard. The results of FNAB were correlated with patients' follow-up. Statistical analysis was done by χ^2-test.

RESULTS

Common complaints of patients with hepatic masses were abdominal pain, anorexia, weight loss and abdominal mass. Patients' age ranged from 1 to 80 years. There were 92 males (61.33%) and 58 females (38.66%). Malignant lesions were common between 40 and 70 years whereas benign were in the age group of 20-40 years. Out of the 150 cases, 5 cases (3.3%) were benign, 142 cases (94.66%) were malignant and 3 cases (2%) were inflammatory. Cytology samples were unsatisfactory for evaluation in three cases (2%). One case contained only a few scattered hepatocytes and blood that turned out to be hemangioendothelioma (1 case) on FNAB. The other two cases, which were PD-HCC (1 case) and metastatic poorly differentiated carcinoma (1 case) on FNAB, showed a necrotic material only. Unsatisfactory FNAB samples (6 cases; 4.02%) showed predominantly necrotic and inflammatory cells. These cases were diagnosed as PD-HCC (2 cases), metastatic adenocarcinoma (2 cases), metastatic poorly differentiated carcinoma (1 case) and metastatic squamous cell carcinoma (1 case) on FNAB. One case of both HCC and metastatic poorly differentiated carcinoma gave unsatisfactory results on both FNAC and FNAB. On follow-up, correct diagnosis was achieved. The diagnostic yield of FNAC and FNAB was 98% and 95.97%, respectively [Tables 1 and 2].

Benign lesions included vascular tumors (2 cases) and hepatic adenoma (HA) (3 cases). Two out of five benign lesions were correctly diagnosed by cytology (diagnostic sensitivity: 40%). One case of hemangioma showed occasional benign spindle endothelial cells [Figure 1a and b]. In correlation with radiology findings diagnosis of hemangioma was made. FNAB confirms the diagnosis of hemangioma [Figure 1c]. Another case showed only benign hepatocytes without definite diagnosis on FNAC, whereas FNAB suggested infantile hemangioendothelioma [Figure 1d and e]. Out of three cases of HA, only one was reported correctly by cytology. It revealed only benign hepatocytes without bile duct cells. The other two cases were misdiagnosed as WD-HCC and focal nodular hyperplasia on cytology. The combined diagnostic sensitivity of FNAC and FNAB was 100% [Tables 1 and 2].

The 142 malignant lesions included hepatoblastoma (1 case), HCC (41 cases) and metastatic tumors (100 cases). The diagnostic sensitivity of FNAC and FNAB for the malignant lesions was 87.32% and 97.18%, respectively [Table 1]. The results of the cyto-histological discrepancies of malignant hepatic masses are given in Table 2. One case of hepatoblastoma was correctly diagnosed by cytology (100%). It showed predominant fetal differentiation of hepatocytes with vague trabecular arrangement of cells [Figure 2]. Ultrasound showed 33 solitary and eight multifocal masses of HCC. The largest and smallest masses measured 18 cm × 19 cm and 1 cm × 1 cm, respectively. A total 35 of the 41 cases of HCC were correctly picked up by FNAC (diagnostic sensitivity: 85.36%). Cytologically, HCC were classified into well- (12 cases; 34.28%), moderately (18 cases; 51.42%) and poorly differentiated types (5 cases; 14.28%). The main cytological features of the WD-HCC were high cellularity with broad trabeculae of large polygonal hepatocytes, increased nucleus-to-cytoplasm (N:C) ratio, a central round nucleus, intranuclear inclusions, abundant granular eosinophilic cytoplasm, intracytoplasmic bile, endothelial

Table 1: Distribution of various hepatic masses in accordance to FNAC and FNAB

Diagnosis	Number of cases correctly diagnosed by FNAB (sensitivity/true positive) (%)	Number of cases correctly diagnosed by FNAC (sensitivity/true positive) (%)	Number of cases incorrectly diagnosed by FNAC (false negative), including unsatisfactory cases	Number of cases in which FNABs were unsatisfactory for evaluation
Benign group (05) (3.3%)				
Vascular lesion (02)	02/02 (100)	01/02 (50)	01 unsatisfactory	00
Hepatic adenoma (03)	03/03 (100)	01/03 (33.33)	02	00
Total	05/05 (100)	02/05 (40)	03/05 (60.0%)	00
Malignant group (142) (94.67%)				
Primary hepatic lesions (28.0%)				
Hepatoblastoma (01)	01/01 (100)	01/01 (100)	00	00
Hepatocellular carcinoma (41)	39/41 (95.12)	35/41 (85.36)	06/41 (14.63%) (included 01 unsatisfactory)	02
Metastatic hepatic lesions (66.67%)				
Adenocarcinoma (75)	73/75 (97.33)	70/75 (93.33)	05/75 (6.66%)	02
Small-cell carcinoma (10)	10/10 (100)	09/10 (90)	01	00
Poorly differentiated carcinoma (08)	07/08 (87.50)	04/08 (50)	04 (included 02 unsatisfactory)	01
Carcinoid tumor (03)	03/03 (100)	02/03 (66.66)	01	00
Malignant melanoma (02)	02/02 (100)	01/02 (50)	01	00
Non-Hodgkin's lymphoma (01)	01/01 (100)	01/01 (100)	00	00
Squamous cell carcinoma (01)	00/01 (00)	01/01 (100)	00	01
Total	138/142 (97.18)	124/142 (87.32)	18/142 (12.67%)	06
Inflammatory group (03) (2.0%)				
Hepatic abscess (02)	02/02 (100)	02/02 (100)	00	00
Hydatid cyst (01)	00/00 (00)	01/01 (100)	00	01 (not done)
Total	02/03 (66.66)	03/03 (100)	00	00
Total (A+B + C)	143/149 (95.77)	129/150 (86)	21/150 (14%)	06/149 (4.02%)

FNAB: Fine-needle aspiration biopsy, FNAC: Fine-needle aspiration cytology

Table 2: Cyto-histological discrepancies of malignant hepatic masses

Histological diagnosis	Number of cases	Cytological diagnosis	Number of cases
WD-HCC	14	WD-HCC	12
		Reactive hepatocytes	01
		Dysplastic hepatocytes	01
MD-HCC	18	MD-HCC	18
PD-HCC	09	PD-HCC	05
		Metastatic adenocarcinoma	03
		Metastatic poorly differentiated carcinoma	01
Metastatic adenocarcinoma	75	Metastatic adenocarcinoma	70
		PD-HCC	03
		Metastatic poorly differentiated carcinoma	02
Metastatic melanoma	02	Metastatic melanoma	01
		PD-HCC	01
Metastatic small-cell carcinoma	10	Metastatic small-cell carcinoma	09
		Metastatic carcinoid tumor	01
Metastatic carcinoid tumor	03	Metastatic carcinoid tumor	02
		Metastatic adenocarcinoma	01

rimming, transgression of vessels through cell clusters and bare atypical nuclei [Figure 3a-c]. Moderately differentiated HCC (MD-HCC) showed many features of WD-HCC. Endothelial rimming, transgressing vessels, eccentric nuclei, multi-nucleation, multiple nucleoli and macronuclei were more common in MD-HCC [Figure 4a-c]. Diffuse clear-cell change was also evident in one case [Figure 5a-c]. PD-HCC showed sheets, small groups and singly dispersed cells. Anisocytosis, anisonucleosis, irregular hyperchromatic nuclear chromatin, multiple nuclei, macronucleoli and bare atypical nuclei were common. A transgressing endothelium, inflammation, necrosis and giant cells were seen in few of cases.

Metastatic tumor was the most common malignant hepatic lesion (66.66%). A total 88 out of 100 cases of metastatic tumors were correctly diagnosed by cytology (diagnostic sensitivity: 88%). Metastatic adenocarcinoma was the commonest type (75 cases) followed by small-cell carcinoma (10 cases), poorly differentiated carcinoma (8 cases), carcinoid

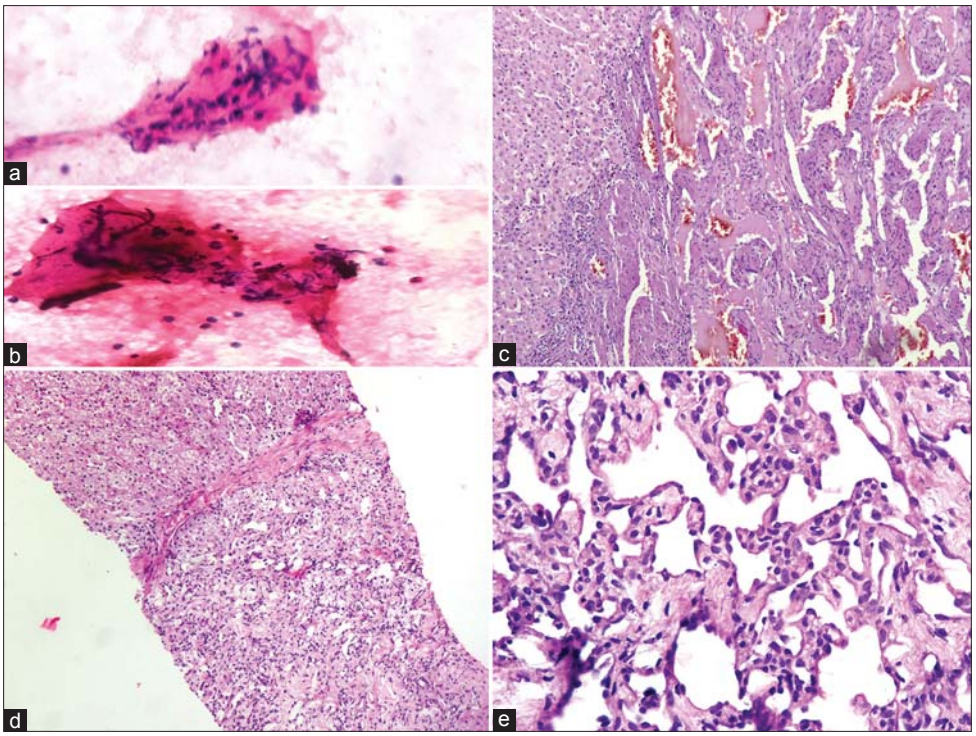

Figure 1: Hemangioma: Fine-needle aspiration cytology: (a and b) Clusters of benign spindle endothelial cells on a background of blood (H and E, ×400). Fine-needle aspiration biopsy: (c) blood-filled spaces are lined by bland endothelial cells with adjacent benign hepatocytes (H and E, ×100). Hepatic hemangioendothelioma: FNAB: (d) Well-demarcated lobular vascular structure with adjacent benign hepatocytes (H and E, ×100). (e) Multiple interconnecting vascular channels of various sizes are lined by a single layer of flat or plump endothelial cells (H and E, ×400)

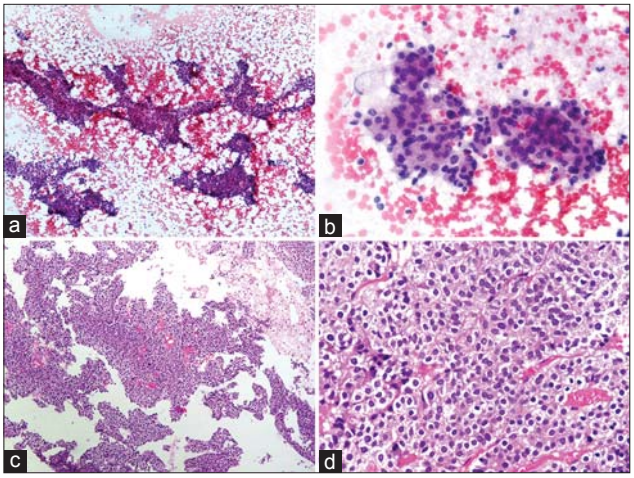

Figure 2: Hepatoblastoma: Fine-needle aspiration cytology: (a) Sheets and clusters show a vague trabecular arrangement of tumor cells (PAP, ×100). (b) cells show round nucleus, granular nuclear chromatin with occasional small distinct nucleoli and moderate granular cytoplasm with an ill-defined border (PAP, ×400). Fine-needle aspiration biopsy: (c) Sheets of fetal hepatocytes in a trabecular pattern (H and E, ×100). (d) Fetal hepatocytes show mild pleomorphism with rounded ovoid nuclei, occasional small distinct nucleoli and perinuclear cytoplasmic clearing (H and E, ×400)

tumor (3 cases), malignant melanoma (2 cases), squamous cell carcinoma (1 case) and non-Hodgkin's lymphoma (1 case). The primary sites of adenocarcinoma were the gastrointestinal tract (24), lung (13), pancreas (7), gall bladder (5), ovary (5), breast (4), prostate (2) and cervix (2) in decreasing order of frequency. Seventy out of 75 cases (93.33%) of adenocarcinoma were correctly diagnosed by cytology [Table 1]. In 13 cases, origin of adenocarcinoma could not be determined on histology. Prediction of primary sites of metastatic disease was possible with clinical and radiological correlation. The common cytological features of adenocarcinoma were high cellularity, columnar or cuboidal tumor cells with mild-to-moderate pleomorphism, high nuclear-to-cytoplasmic (N:C) ratio with a central or eccentrically placed nucleus, fine dispersed-to-coarse chromatin, and scanty to moderately vacuolated or granular eosinophilic cytoplasm. Cells were arranged in glands, acinar or palisade-liked patterns; three-dimensional clusters; or singly. Inflammation, necrosis and fibrosis were prominent in some cases. Transgressing vessels through tumor cell clusters were also evident in two cases [Figure 6a-c].

Small-cell carcinoma showed small monomorphic cells with finely granular nuclear chromatin, inconspicuous or absent nucleoli, and scanty cytoplasm. The tumor cells were non-cohesive and a few arranged in loose clusters. Nuclear molding and smearing artifacts were also evident. Mitotic activity was not seen [Figure 7a-d]. Carcinoid tumor revealed fairly uniform small-sized, more cohesive cells with abundant, better defined intact cytoplasm, finely stippled nuclear chromatin and small nucleoli. Mitotic activity and necrosis were not evident. Metastatic poorly differentiated carcinoma showed large pleomorphic cells with hyperchromatic multi-lobulated nuclei and scanty-to-moderate cytoplasm. The original nature of cells

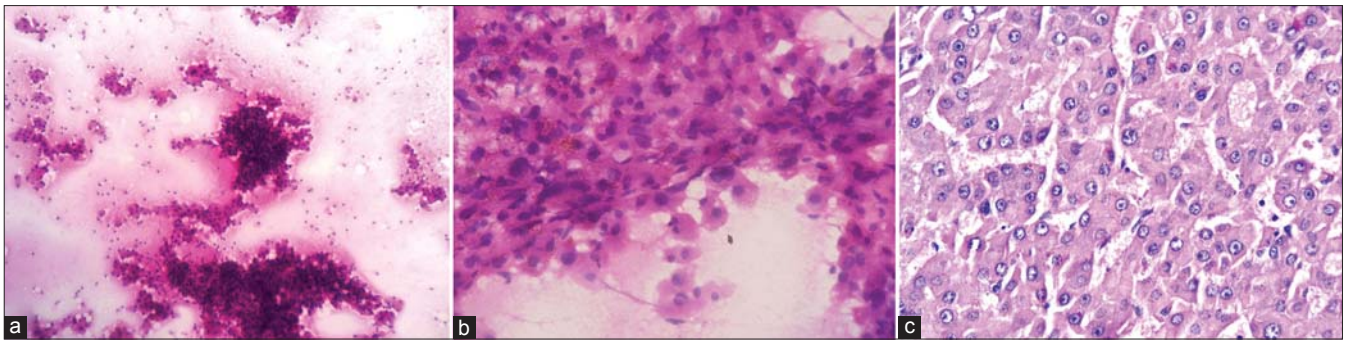

Figure 3: WD-HCC: Fine-needle aspiration cytology: (a) High cellularity with large clusters of hepatocytes and many bare atypical nuclei (H and E, ×100). (b) Polygonal hepatocytes show increased nucleus-to-cytoplasmic (N:C) ratio, rounded nuclei, abundant granular eosinophilic cytoplasm and intracytoplasmic bile; cluster of malignant cells traversed by spindle endothelial cells (H and E, ×400). Fine-needle aspiration biopsy: (c) Hepatocytes show central nuclei, prominent nucleoli and granular cytoplasm; cells show a predominant trabecular pattern with occasional acinar configuration (H and E, ×400)

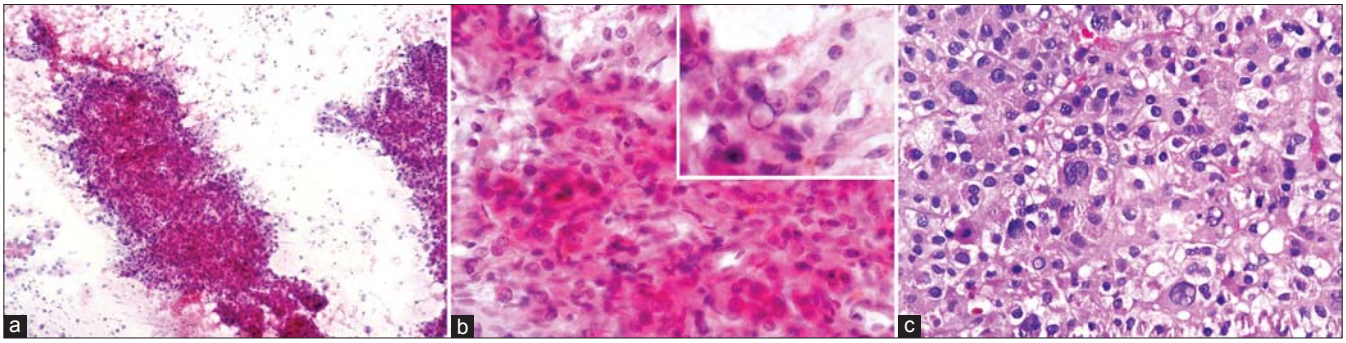

Figure 4: MD-HCC: Fine-needle aspiration cytology: (a) Large clusters of malignant hepatocytes show a vague trabecular pattern with many dispersed atypical nuclei (PAP, ×100). (b) Hepatocytes show a high N:C ratio, large nuclei, multi-nucleation, macronucleoli and golden yellow bile thrombi. The inset figure shows a large intranuclear inclusion (PAP, ×400). Fine-needle aspiration biopsy: (c) Pleomorphic hepatocytes show multi-nucleation, prominent nucleoli, intranuclear inclusions and a focal area of cytoplasmic clearing (H and E, ×400)

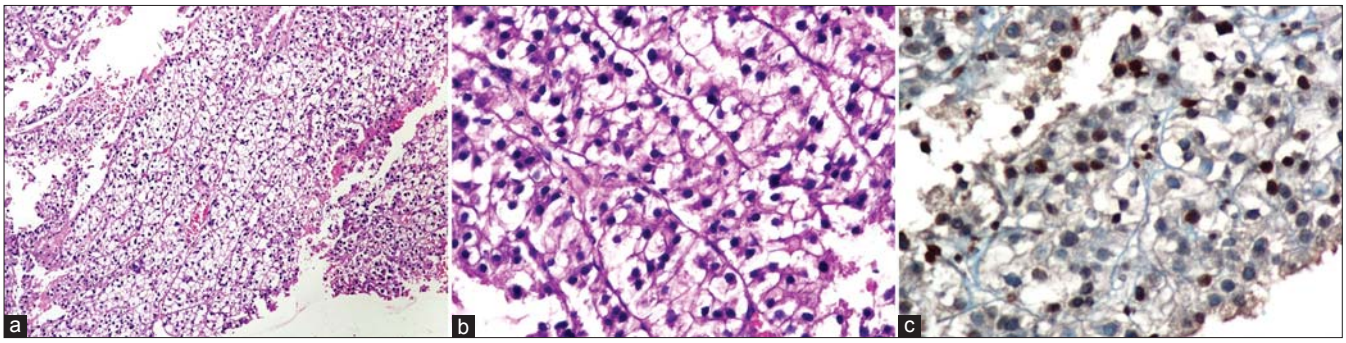

Figure 5: Clear-cell HCC: Fine-needle aspiration biopsy: (a) Sheets of clear-cell hepatocytes (H and E, ×100). (b) Trabecular pattern of hepatocytes showing extensive cytoplasmic clearing and large hyperchromatic nuclei (H and E, ×400). (c) Positive immunostaining for AFP (×400)

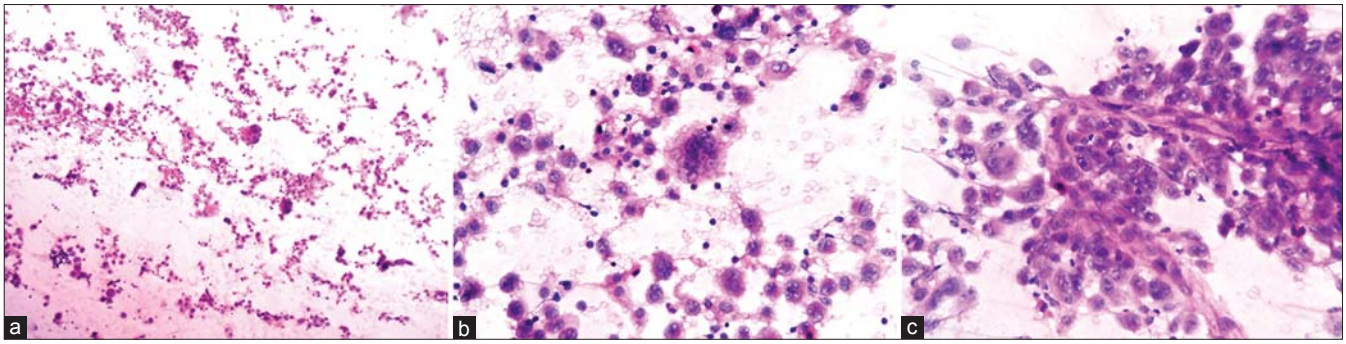

Figure 6: Metastatic adenocarcinoma: Fine-needle aspiration cytology: (a) Loose aggregates with dispersed tumor cells on a necrotic and inflammatory background (H and E, ×100). (b) Tumor cells showing marked pleomorphism, a high N:C ratio, a central to eccentrically placed vesicular nucleus, multi-nucleation, prominent nucleoli and scanty-to-moderate cytoplasm. A few tumor giant cells are also evident (H and E, ×400). (c) Cluster of tumor cells traversed by spindle endothelial cells (H and E, ×400)

cannot be assessed. Metastatic melanoma showed large tumor cells with abundant well-defined cytoplasm, multiple nuclei with prominent nucleoli and intranuclear cytoplasmic inclusions. In one case, melanin was not found in metastatic lesions and mimicked HCC. Non-Hodgkin's lymphoma showed dispersed monotonous cells with granular nuclear chromatin and scanty cytoplasm [Figure 8a-d]. Squamous cell carcinoma showed squamoid, tadpole-like cells with well-defined, abundant, keratinized cytoplasm and irregular hyperchromatic nuclei.

Statistical analysis showed that the cytological diagnostic sensitivity for the benign and malignant tumors was 40% and 87.32%, respectively. This difference was statistically significant ($P < 0.05$). FNAB showed a statistically significant difference ($P < 0.05$) compared with FNAC in the diagnosis of benign and malignant hepatic masses [Table 3].

Inflammatory lesions comprised hepatic abscess (2 cases) and a hydatid cyst (1 case). Pyogenic hepatic abscesses showed numerous neutrophils and necrosis on cytology [Figure 9a and b]. Similar findings were seen on FNAB. The hydatid cyst by the larvae of *Echinococcus granulosus* revealed diagnostic scolices, hooklets and a laminated membrane along with a few hepatocytes on cytology [Figure 9c and d]. FNAB was not performed in the case of the hydatid cyst due to risk of cyst rupture. Inflammatory lesions were correctly reported on FNAC with 100% diagnostic sensitivity [Table 1].

DISCUSSION

Tissue diagnosis of hepatic masses are very important for management.[1,2] Focal hepatic lesions range from cysts and inflammatory processes to neoplasms, be they benign or malignant, primary or metastatic.[3] Clinical, radiological and serological findings cannot reliably distinguish a benign from a malignant lesion, but they can help to narrow the differential diagnosis.[1-3] In such instances, FNAC under image guidance has gained increasing acceptance as the diagnostic procedure of choice.[1-7,11] Assistance of a cytopathologist during the procedure increases overall accuracy.[2] The contraindications of FNAC are hemorrhagic diathesis, prolonged prothrombin time, vascular structure in the path and suspected extrahepatic obstructive jaundice.[2,4,5] Suspected hemangioma is not considered an absolute contraindication. However, aspirating hemangioma carries a low risk of hemorrhage particularly when large needles are used.[2,4,11] A clinically suspected hydatid cyst is a contraindication for FNAC because of the risk of a fatal anaphylactic reaction. However, no major complications have been reported even when hydatid cysts are inadvertently aspirated like in our case.[1,2,4] According to our study, ultrasound guidance is usually preferred for its simplicity, real-time monitoring and flexible needle placement. CT guidance is expensive and time-consuming so it is reserved for lesions that are not demonstrated by ultrasound.

Hemangiomas, common benign tumors of the liver, are often asymptomatic and detected incidentally. Characteristic benign spindle endothelial cells and fragments of fibrovascular tissue on cytology may not be obvious like in our case. In such cases, radiologic imaging is often essential and diagnostic for hemangioma.[2,12] Many times benign hepatocellular neoplasms such as HA and focal nodular hyperplasia can be difficult or impossible to diagnose on FNAC alone because of their cytologic similarities to normal liver, cirrhosis or well-differentiated HCC. Atypia may be seen in HA and it may represent a dysplastic process.[2] The recognition of polymorphism with variation of cell and nuclear size, and a normal N:C ratio of 1:3 should alert one to the likelihood of benignity of the hepatocytes.[3] In our case, markedly reactive atypical hepatocytes of HA misled as diagnosis of WD-HCC on cytology alone. In such instances, FNAB is essential for architectural evaluation.[2] Cytologically, focal nodular hyperplasia contains bland hepatocytes with bile duct epithelium and stromal fragments. HA characteristically contains hepatocytes only.[2] Bland hepatocytes with occasional bile duct epithelium lead to a misdiagnosis of focal nodular hyperplasia in our case. Bile duct epithelium may have been extracted from tissue adjacent to adenoma. So it is crucial that the needle must be within the lesion and only the lesion is sampled.

Hepatoblastoma usually affects 3-year-old or younger children and has markedly elevated serum α-fetoprotein (AFP) levels. Hepatoblastoma is not associated with cirrhosis. Hepatoblastoma exhibits various patterns of differentiation, including fetal, embryonal and undifferentiated small cells, and macrotrabecular types, as well as varying amounts of mesenchymal components.[2] On FNAC, a hepatoblastoma can resemble a normal liver if it exhibits a predominantly fetal-type differentiation with a trabecular pattern like in our case. If other epithelial components such as embryonal, small-cell or macrotrabecular patterns are present, the tumor shows a more heterogeneous population of variably sized cells with or without trabecular groups, suggesting diagnosis of hepatoblastoma. On cytology smears alone, abundant embryonal or small-cell components may resemble other small-cell tumors of childhood, such as embryonal rhabdomyosarcoma, neuroblastoma, Ewing's sarcoma, Wilms' tumor and lymphoma. The macrotrabecular component can be more cytologically pleomorphic, mimicking trabecular HCC.[2] Pure fetal differentiation is associated with improved survival when compared with other histologic patterns of hepatoblastoma.[13]

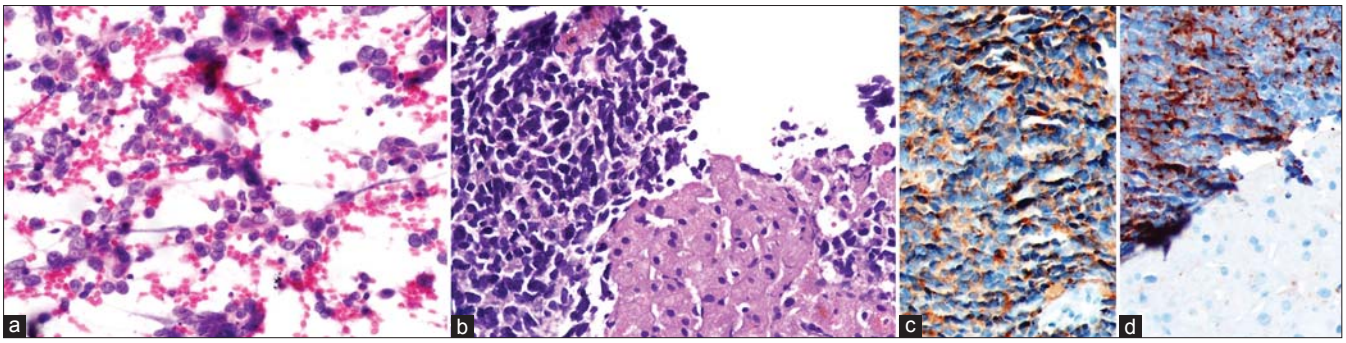

Figure 7: Metastatic small-cell carcinoma: Fine-needle aspiration cytology: (a) Loose aggregates with dispersed small monomorphic cells with scanty cytoplasm, finely granular nuclear chromatin and inconspicuous nucleoli. Nuclear molding and smearing artifacts are evident (PAP, ×400). Fine-needle aspiration biopsy: (b) Fragments of small round tumor cells with adjacent benign hepatocytes (H and E, ×400). Positive immunostaining for (c) chromogranin (×400) and (d) synaptophysin (×400)

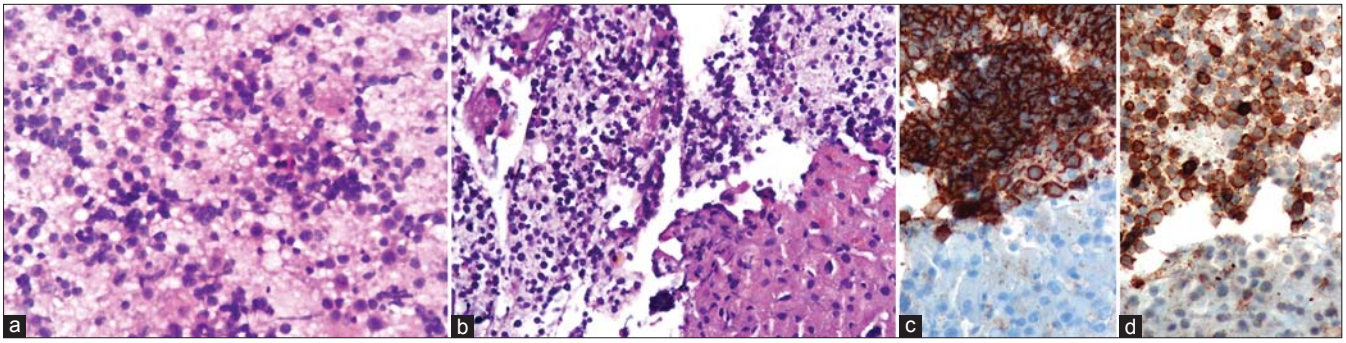

Figure 8: Non-Hodgkin's lymphoma: Fine-needle aspiration cytology: (a) Dispersed large lymphoid cells showing pale nuclei, multiple nucleoli and scanty cytoplasm (H and E, ×400). Fine-needle aspiration biopsy: (b) Fragments of lymphoid tumor cells and adjacent sheets of benign hepatocytes (H and E, ×100). Positive immunostaining for (c) leukocyte common antigen (×400) and (d) CD20 (×400)

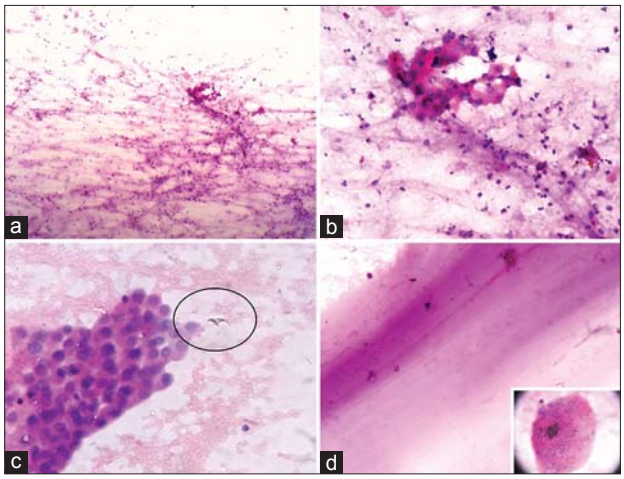

Figure 9: Pyogenic hepatic abscesses: Fine-needle aspiration cytology: (a) Numerous neutrophils and necrotic debris (H and E, ×40). (b) Hepatocytes showing mild atypia and bile pigments on an inflammatory background (H and E, ×400). Hydatid cyst: FNAC: (c) Refractile hooklets with adjacent hepatocytes (H and E, ×400). (d) A fragment of a laminated membrane; the inset figure shows full scolices with refractile hooklets (H and E, ×400)

Tables 3: Comparison of the diagnostic rate of FNAC and FNAB in benign and malignant hepatic masses

Diagnostic procedure	Benign cases	Malignant cases	χ^2-Value	*P* value
FNAC	02/05 (40%)	124/142 (87.32%)	8.834	0.002
FNAB	05/05 (100%)	138/142 (97.18%)	0.145	0.703
χ^2-value	4.286	9.657	–	–
P value	0.0384	0.0018	–	–

FNAB: Fine-needle aspiration biopsy, FNAC: Fine-needle aspiration cytology

HCC can be small and focal, solitary and large, multifocal or diffuse, and infiltrating, thereby, mimicking benign lesions on one hand and metastases on the other, especially in imaging studies. Serum AFP, though fairly specific, has poor sensitivity for the diagnosis of HCC, regardless of tumor size or differentiation.[3] The cytological appearance of HCC varies with the degree of differentiation.[2] We classify HCC into well, moderately and poorly differentiated types based on the features described by Swamy *et al*., and Wee *et al*.[1,14] In our study, cyto-diagnosis of MD-HCC was usually straightforward because they showed malignant features with residual hepatic differentiation. Difficulties in cytological diagnoses arise in the well and poorly differentiated ends of the spectrum of HCC. Sometimes, WD-HCC can closely resemble benign or reactive conditions such as HA, macro-regenerative nodule, dysplastic nodule, chronic hepatitis or cirrhosis.[4,6,7,11] On the other hand, cytology of benign lesions may show significant reactive atypia or even dysplasia mimicking WD-HCC.[4,6] The most useful criteria to separate highly WD-HCC from reactive hepatocytes/cirrhosis are hypercellularity, cohesive broad

trabeculae (>2-cell-thick), small monotonous hepatocytes with nuclear crowding, increased N:C ratio, cytoplasmic hyaline inclusions, atypical naked nuclei, macronucleoli, tumor giant cells, and a transgressing or peripheral endothelium. Absence of bile epithelium favors HCC.[1,4,6,14] In our case, hepatocytes showed minimal atypia. The cell cords were not >2-cell-thick and subtle increases in the N:C ratio may not have been appreciated by light microscopy. Definitive diagnosis of a dysplastic nodule or reactive hepatocytes, and exclusion of WD-HCC often requires microhistology section by FNAB. The inherent difficulty of cytology in distinguishing small/early/well-differentiated HCC from benign hepatocellular nodular lesions frequently leads to indeterminate reports. The histopathological interpretation of small suspicious nodules is highly controversial and necessitates refinement of current histopathologic criteria for diagnosis of small ("early") HCC.[3] Ultrasound-guided biopsy can be successfully used as a first-step diagnostic tool, even for nodules <10 mm in diameter and is often the only way to differentiate between benign and malignant nodules in a cirrhotic liver.[1] As early diagnosis and treatment of HCC carry good prognosis, we recommend ultrasound-guided FNAC and FNAB from such small lesions as soon as possible. If adjacent benign material predominates in the FNAC specimen, the tumor population may be missed. In such instances, FNAB for microhistology provides architectural details, which increase diagnostic accuracy. The sensitivity of FNAC for HCC was 85.36%, with a 14.63% false-negative rate, in our study compared with 96% sensitivity in the study by Nazir *et al*.[4] FNABs are unsatisfactory in large necrotic HCC whereas FNACs are unsatisfactory in small nodules due to sampling errors.

The most important cytological features of HCC are the trabecular pattern of hepatocytes (>2-cell-thick), irregular granular chromatin, multiple nucleoli, intracytoplasmic bile and atypical naked nuclei.[1,3,5,8] Increased N:C ratio is the single most important feature favoring malignant hepatocytes.[1,3,8,10] Intracytoplasmic eosinophilic inclusions strongly support HCC. They have also been reported in ovarian, breast, lung and adrenal gland tumors, and in asbestosis lung.[1] Intranuclear cytoplasmic inclusions are evident in all groups. However, they are not diagnostic of a benign or malignant process. Iron and lipochrome pigments within hepatocytes are nearly always associated with benign processes. HCC can contain fat, bile or Mallory's hyaline, so the presence or absence of these features is of no help in distinguishing benign from malignant lesions, but only helps in supporting the hepatic origin.[3] The presence of characteristic endothelial patterns is an important feature of WD-HCC. The basketing pattern consists of groups or trabeculae of hepatocytes wrapped by endothelial cells. This pattern is specific but observed only in 50% of HCC. It is often absent in PD-HCC. The pattern is seldom seen in benign hepatic lesions or other malignancies.[1-3] The other endothelial pattern consists of traversing capillaries through groups of hepatocytes. This pattern is noted in over 90% of HCC but is less specific since it can be seen in other malignancies and rarely in some non-neoplastic liver conditions.[1-3,10] Focal clear-cell changes are frequent. Diffuse clear-cell changes occur in <10% of cases of HCC. Diffuse clear-cell change is not diagnostic of malignancy, but, when present in a significant amount, can help to diagnose HCC. Clear-cell malignancy can arise in the kidney, adrenal and ovary.[3] The frequency of anisocytosis, anisonucleosis, eccentric nuclei, multiple nucleoli and macronuclei, irregular nuclear contours, increased chromatin density, atypical naked hepatocytic nuclei and cellular dissociation is increased with higher grade of HCC.[1,3,10] Fibrolamellar HCC occurs in non-cirrhotic liver and has good prognosis. It comprises large, dyscohesive, polygonal hepatocytes with abundant oncocytic cytoplasm and lamellar fibrosis. Pale bodies are common.[3] Our study concludes that all (as many as possible) of the cytological features of HCC should be considered together to increase diagnostic sensitivity, rather than considering one or two features alone, even if they are important ones.

FNAC is being increasingly used for diagnosis of liver metastasis with excellent results.[6,9] The most common tumor in our series was metastatic carcinoma, especially adenocarcinoma. Metastatic adenocarcinomas usually show variable differentiation. The cytoplasm differs markedly from that of hepatocytes.[1-3,5] Necrosis, inflammation, mucin, and residual columnar or cuboidal differentiation favor metastatic adenocarcinoma. Necrosis is more abundant in metastatic adenocarcinoma of colon than HCC. Adenocarcinoma of the breast only rarely metastasizes to the liver before the primary disease is discovered. A strong morphological similarity occurs between PD-HCC, metastatic poorly differentiated carcinoma and metastatic adenocarcinoma, which leads to diagnostic difficulty on cytology alone. PD-HCC shows obvious malignant features, but the hepatocytic origin of the cells may not be clear.[2,3] Das[15] has stated that the gold standard for cytological diagnosis of metastatic deposits remains identification of malignant cells of non-hepatocytic origin. The presence of large three-dimensional clusters, glandular differentiation, benign hepatocytes along with clusters and dissociated, highly pleomorphic malignant cells suggests metastatic poorly differentiated carcinoma over PD-HCC.[1,3,5] Accurate detection of metastases, especially unresectable lesions, is necessary for appropriate therapy.[3] Bottles *et al*., showed centrally placed nuclei, malignant cells separated by sinusoidal capillaries and bile as the key cytologic criteria for HCC. Endothelial cells surrounding tumor cell clusters and intranuclear cytoplasmic inclusions were selected as the secondary criteria.[9] Unfortunately bile is present in only

half of the cases.[2] In our case, a transgressing endothelium and intranuclear inclusions of metastatic carcinoma are responsible for the misdiagnosis as PD-HCC. Here, FNAB is important to differentiate PD-HCC from metastases. Our study shows a cytological diagnostic sensitivity of 88%, with a false-negative rate of 12% for liver metastasis. One of the most challenging problems is to suggest the occult primary sites of tumors. The first step is to identify and classify tumor cells into the broad categories of adenocarcinoma, squamous cell carcinoma, melanoma, carcinoid tumor, lymphoma, poorly differentiated carcinoma or sarcoma. When an adenocarcinoma is encountered, distinction between a primary and metastasis becomes a serious task, and in many cases impossible. Recognition of metastatic adenocarcinoma in liver cytology is usually easy, if the tumor is well- to moderately differentiated-type. Next, if the pathologist is informed about the original disease and has access to previous material, the diagnosis of metastatic disease is usually straightforward. If the liver mass is the only known lesion, it might be an HCC or metastatic tumor. Such instances may present a diagnostic problem for the pathologist, especially when the neoplasm is poorly differentiated-type. A markedly elevated serum AFP level and the finding of a single lesion with or without satellite lesions on imaging favor a primary tumor over metastatic disease. HCC has deficient or absent reticulin. It enhances diagnostic accuracy, particularly for WD-HCC.[3,10,16] Immunocytochemistry is of little help in differentiating PD-HCC from metastatic disease because of lack of highly specific markers.[2] Positive AFP staining is reported in 40% of HCC, but negative staining does not exclude diagnosis of HCC.[2,3] A canalicular staining pattern of antibodies against polyclonal carcinoembryonic antigen and diffuse positive staining with endothelial cells markers (such as CD34, factor VIII) can help to distinguish HCC from metastatic adenocarcinoma.[2,3] But positive staining is least often identified in PD-HCC. CD10 is expressed in normal and neoplastic liver. Although it does not differentiate between benign and malignant hepatocellular lesions, CD10 is very useful in distinguishing HCC from non-HCC malignancies.[3] Cytokeratin (CAM) 5.2 is the most reliable cytokeratin antibody for HCC. AE1/AE3 negativity is expected in hepatocellular lesions.[3] HepPar1 has been shown to be quite specific and a sensitive marker for HCC. About 83-100% of HCC stain positive with HepPar1, but only 4-15% of metastatic carcinomas are positive. Unfortunately, only 56% of PD-HCC express Hep Par1.[2,3] In our view, strict clinico-radio-pathological correlation is the first step toward treatment.

Metastatic melanoma may present diagnostic difficulty with HCC, especially when the primary has not been discovered. Melanoma has several features in common with HCC, including polygonal cells with centrally placed nuclei, prominent nucleoli and intranuclear cytoplasmic inclusions. Presence of brown granules of melanin has been considered an important diagnostic feature of melanoma. Even melanin pigment may resemble various liver cell pigments. Unfortunately melanin is usually not found in metastatic lesions, as also in our case.[6] Immunocytochemistry for HMB-45, S-100 protein and cytokeratin is recommended.[6] Metastatic squamous cell carcinoma, usually from the lung, may not pose any diagnostic difficulty except for poorly differentiated tumors, in the absence of keratin and in the presence of marked necrosis with inflammation.[1,3,5] Small/intermediate round-cell malignancies include neuroendocrine tumors, small-cell undifferentiated carcinomas and lymphomas.[3] Most neuroendocrine tumors are from the gastrointestinal tract, pancreaticobiliary tract or lung. A primary hepatic neuroendocrine tumor is unusual. Small-cell undifferentiated carcinoma usually arises from the lung. Lymphoma seldom presents as a primary neoplasm, although hepatic involvement is common in advanced disease. It can be mistaken for poorly differentiated carcinoma or HCC.[3,5] In our study, hemorrhage, low cellularity, loose cohesive clusters due to smearing artifact, drying artifact and poor spread led to the misdiagnosis of small round-cell tumors. Pleomorphic cell malignancies include large-cell undifferentiated carcinomas, large-cell lymphomas, germ-cell tumors and various sarcomas.[3] Spindle-cell malignancies include leiomyosarcoma, neurogenic tumors, malignant fibrous histiocytoma, undifferentiated sarcoma and fibroblastic/stromal tumors, including gastrointestinal stromal tumor. Sarcomatoid HCC or cholangiocarcinoma with a spindle-cell component has to be considered in such instances.[3] Hepatoid carcinoma usually arises in the lung and gastrointestinal tract. It has a tendency for vascular permeation and distant metastases. It produces AFP and mimic HCC.[3]

The diagnostic sensitivity of FNAC in our study was 86%, with a diagnostic yield of 98% compared with 90% and 83.4%, respectively, in the study by Rasania *et al*.[5] Our results are comparable with other studies such as Kuo *et al*.,[16] (86.1%), Tsai *et al*.,[17] (88.7%), Cochand-Priollet *et al*.,[18] (82.6%) and França *et al*.,[19] (78%). The sensitivity of FNAC for hepatic malignancy was 99.5% and 95.3% in the study by Soyuer *et al*.,[20] and Nazir *et al*.[4] The diagnostic sensitivity of FNAC for malignant and benign hepatic lesions was 87.32% and 40%, respectively, in our study. This difference is statistically significant. Diagnosis on FNAC is easier in malignant hepatic lesions than benign lesions, and avoids unnecessary diagnostic laparotomies. FNAC has been reported to be a rapid, safe, minimally invasive, accurate and cost-effective technique for diagnosis of hepatic masses. Abundant well-prepared material and thorough screening of smears, combined with relevant clinical, radiologic and serologic studies,

are the key features to increase the diagnostic accuracy of FNAC. However, FNAC cannot serve as an exclusive diagnostic method for malignant lesions due to its 14.0% false-negative rate. An improved diagnostic sensitivity of 98.66% is achieved with a combination of cytologic and histologic results compared with 86% by Cochand-Priollet *et al*.,[18] 88% by França *et al*.,[19] 80% by Herszenyi *et al*.,[21] and 98% by Sanglli *et al*.[22] In our study, FNAB showed 95.77% sensitivity similar to 93% by Herszenyi *et al*.[21] Our study favors FNAC in combination with FNAB as a minimally invasive diagnostic procedure for hepatic masses as both are complimentary to each other and increase diagnostic sensitivity. However, the final choice should be based on the provisional clinical diagnosis, personal experience and expertise. The wide application of molecular biology techniques has made it possible to detect nucleic acid and various kinds of oncogenes even in a few cells on FNAC as well as on FNAB. Therefore, molecular biology biopsies for *in situ* hybridization and polymerase chain reaction are the future hot points of FNAC.[11]

Complications of hepatic FNAC are rare with about 0.5% minor complications, 0.05% major complications requiring surgery and less than 0.01% mortality.[11] In our study, the core biopsy technique was not associated with an increased complication rate similar to a previous study.[18] Complications include hemorrhage, bile leakage, sepsis, pneumothorax, hypotension and pancreatitis. In our study, complications were limited to hemorrhage and a mild degree of pneumothorax. The frequency of complications is often related to the vascularity and location of the lesions, the diameter of the needle and the number of passes.[2,3,23] A single pass with larger bore needles (<20 Gauge) may be preferable to multiple passes by finer needles, to obtain sufficient material for cytohistologic examination.[3] The risk of malignancy growing along the biopsy tract is small but real, with a reported incidence up to 1:1000 in abdominal biopsies (0.003-0.009%).[23]

CONCLUSION

Tissue diagnosis is recommended for focal hepatic lesions as the risk of aggressive therapy is greater than the risk of a minimally invasive diagnostic procedure. Ultrasound or CT scan-guided FNAC is a useful diagnostic procedure for evaluating hepatic masses as the procedure is rapid, simple, cost-effective and safe. FNAC is more accurate for diagnosis of malignant than benign lesions. FNAC has its own limitations and poses a few diagnostic challenges in benign lesions, WD-HCC, PD-HCC, metastatic carcinoma and detection of the primary site of metastatic deposits. In fact, some of these neoplasms may be impossible to diagnose on cytology smears alone, and it is necessary to augment the cytologic analysis with microhistology by FNAB. FNAB allows architectural, cellular and immunohistochemical evaluation. To obtain maximum diagnostic information with reduction of indeterminate reports, a combined approach of FNAC and FNAB with clinical findings, tumor markers and ancillary techniques should be used.

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