

Original Research Article

Ultrasound guided fine needle aspiration cytology of space occupying lesions of liver

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ABSTRACT

Background: The liver is a common site for primary and secondary tumors; most often from malignant tumors within the abdomen and from extra-abdominal primary malignant neoplasm, but also for sarcomas and lymphomas. The main indication of fine-needle aspiration cytology (FNAC) of the liver is diagnosis of single or multiple space occupying lesions. This study aims to evaluate the cyto-morphology of primary and secondary neoplasms of liver and non-neoplastic conditions in the smears of ultrasound guided fine needle aspiration of SOL of liver, to evaluate the cytomorphologic features and to evaluate the erroneous diagnosis when compared with cell block preparation of aspirate (tissue diagnosis).

Methods: This study is hospital-based prospective study including 57 patients with space occupying lesion of the liver mass. FNAC were carried out under the guidance of sonography and/or computed tomography. The staining performed were conventional for smears of the aspirate. The cell blocks were prepared from the aspirates by the established procedures. The values of correlation were bought out.

Results: Males predominated over females. The distribution of cases for cyto-diagnosis were as follows; cirrhosis with hepatic granulosa (3 cases), pyogenic abscess (3 cases), hepatic adenoma (2 cases), Focal nodular hyperplasia (2 cases), hepatoblastoma (1 case), hepatocellular carcinoma (17 cases), adenocarcinoma deposits (20 cases), deposits of small cell carcinoma (3 cases), deposits of ductal carcinoma (2 cases) and 1 case each of deposits of squamous cell carcinoma, non-Hodgkin's lymphoma, neuroendocrine tumor and adeno-squamous carcinoma. Values of correlation were as follows: sensitivity 97.61%, specificity 100%, PPV 100%, NPV 97.82% and diagnostic accuracy of 98.85%.

Conclusions: FNAC is concluded to be first rank diagnostic procedure in diagnosis of SOL of liver of varied etiology with high values of NPV and PPV. It is also concluded that it helps in staging of metastatic malignancies.

Keywords: Cytomorphology, FNAC, Liver SOL, Liver mass, Metastatic malignancy, Malignancy

INTRODUCTION

The liver is a common site for primary and secondary tumors. The secondary tumors are not only the deposits from malignant tumors within the abdomen but also from extra-abdominal primary malignant neoplasm. Liver is also a site for deposits of sarcoma and lymphoma. These lesions usually present as single or multiple space occupying lesions (SOL) in liver.¹ Their diagnostic

evolution holds the key for institution of therapy, prognosis vis-a-vis staging of malignant neoplasm.² The ultrasound guided fine needle aspiration cytology in recent years have paved the way for diagnostic assessment of such SOL of high accuracy, sensitivity and specificity.³⁻⁷ There are areas where fine needle aspiration cytology requires attention in avoidance of false diagnosis. FNAC though have surpassed the necessity of tissue confirmation of SOL, it is still dogged with certain

controversies, challenges and expectations. These too say, a few is due to grey zones and overlap cytomorphology at interpretation.⁸⁻¹³ The commonest of them is to distinguish between Hepatocellular adenoma or Nodular hyperplasia from Well-differentiated HCC, Poorly differentiated HCC from that of metastatic poorly differentiated epithelial malignancies, grading of HCC and suggesting the type and site of origin of tumour for hepatic metastatic lesions.¹⁴⁻¹⁷ The advantage of ultrasound guided fine needle aspiration cytology is that the areas of interest within the liver can be identified and thereby the cellular confounders can be excluded to arrive at specific diagnosis.¹⁸⁻²¹ The study was conducted with the aim of diagnosing ultrasound guided fine needle aspiration cytology of SOL of liver.

METHODS

The present study is a prospective observational study which was carried out in the division of Cytopathology in Department of Pathology, JNMC, Sawangi during period from August 2016 to July 2018. The patients with suspected space occupying lesions of the liver were recorded for their preliminary clinical and personal data.

All these patient underwent

- i) Complete blood count
- ii) Clinical enzymology of liver (ALT, AST, ALP) and bilirubin level
- iii) Ultrasonography examination of abdomen that illustrated the sonographic findings of hepatobiliary system.

Existing known primary malignant neoplasm too was noted. The study was cleared by Institutional Ethics Committee for its performing details. A total of 57 cases were taken with following inclusion and exclusion criteria.²²⁻²⁷ All the cases of SOL diagnosed on USG examination with clinically and ultrasonographically suggestive of neoplastic etiology than the infective one and with known primary suspected of metastasis in liver were included in the study.²⁸⁻³¹ The cases with USG findings of diffuse parenchymatous liver disease suspicious of parasitic cyst such as hydatid cyst or others, with suspected hematoma or hemangioma on USG, with bleeding diathesis as detected on pre-procedural workup, with extreme hyperbilirubinemia with Hepatic encephalopathy and/or Hepatorenal shunts and prolonged Prothrombin time (PT/INR) without prior treatment with Vitamin K were excluded from the study. The baseline blood biochemistry was carried out in all the patients recruited in the study which consisted in each patient was of ALT, AST and ALP and bilirubin estimation along with PT (Prothrombin Time) test. The prolonged PT was considered as contraindication for FNAC of SOL and these patients were treated with Vitamin K and brought back to the FNAC procedure protocol. Under the aseptic

precautions, the FNAC of SOL of liver was carried out as a real time procedure. The procedure was carried out by the guidance using Aloka Arrietta S60 linear and curvilinear probes of frequency 7.5-15MHz and 3.55MHz respectively. The SOL was aspirated 24 no. spinal disposable needle. The visual quantitation of aspirates was performed to be qualified for cyto-smear preparation as well as for submission of cell-block. The physical characters of the aspirates were noted. The first few smears from the aspirates were made with minimum 2 dry fixed smears and 2 wet fixed smears in 95% ethyl alcohol. The other part of the aspirate was transferred to formalin containing tube for the procedure of cell-block preparation of the needle aspirate (needle biopsy tissue). The formalin fixed tissue material of SOL obtained on FNAC was processed for cell block preparation. The sections were cut in 5um thickness and stained by H&E stain by standard methodologies. The diagnosis achieved on tissue-blocks of needle aspirate was considered as gold standard for comparison for cyto-diagnosis achieved on cyto-smears examination. The histopathological diagnosis was performed by using standard references.

RESULTS

A total of 57 patients with space occupying lesions diagnosed on abdominal ultrasonography who met the inclusion criteria of the study were recruited. The maximum patients were in age group of 61-70 years. The youngest patient was of 19 years old and was diagnosed as Infantile Hepatoblastoma and the oldest was 84 years old and diagnosed as metastatic deposits of poorly differentiated adenocarcinoma on fine-needle aspiration cytology of SOL. The male predominated female in a proportion of 2:1. The maximum number of males (n=11) recruited in the present study were in age range of 61-70 years while maximum females (n=8) were in age group of 41-50 years. The highest frequency of clinical diagnosis for SOL was Hepatocellular Carcinoma (HCC). Overall 40 cases were suspected to be malignant clinically. No significant abnormality was observed in these patients for complete blood counts. However, the peripheral smear examination of 23 patients revealed the presence of target red blood cells and 27 patients showed mean corpuscular value over 100 fl. The mean ALT value in the patient recruited in the present study was 30+/-5U/l, for AST 20+/-5U/l and for ALP 80+/-5U/l. Total bilirubin levels in all patients were raised. The lowest one was 2.3mg/dl while highest one was 4.7mg/dl. Direct:Indirect Bilirubin ratio when calculated as a mean over the recruited population was >0.42. AFP values were available in 10 cases of clinically suspected of HCC. The patients had high values of AFP minimally increased by 4 folds upto 6 folds. Ultrasonography of liver revealed multifocal lesions in 66.7% and solitary lesion in 33.3% cases. Maximum SOL observed hypo-echogenicity present in 35 cases (61.41%). Internal calcification was observed in 35 cases (61.39%). Most of the SOLs were deeply seated (73.68%) and were located in right lobe (52.63%) of liver. Size varied between 0.5 to 5 cm in 61.41% of

lesions and associated cirrhosis was seen in 36.84% cases. A total of 42 cases (73.68%) were diagnosed malignant on ultrasonography. Maximum discordance was observed amongst clinically suspected cases of Hepatocellular carcinoma (n=25) which were diagnosed metastatic deposits in 6 cases and nodule of cirrhosis in 2 cases. Ultrasonography diagnosed 23 cases (40.35%) of HCC and 19 cases (33.33%) of metastatic deposits. Suspected liver abscess was diagnosed on 4 cases (7%). No complications of FNAC of SOL of liver which required treatment were observed in all 57 cases. Twelve patients complaint of dull post-procedural pain at right hypochondrium. The needle aspirates of 49 cases were available and were processed by formalin fixation and histokinette to obtain 5u thick sections similar to regular histopathology. Out of 49 paraffin blocks, 46 contained representative material while 3 tissue blocks showed only blood clots on histopathological examination. The

morphology of the sections of needle aspirates for cell block were reported similarly to histopathological examination by standard text. The broad categories of cyto-diagnosis achieved in 57 cases of SOL of liver by FNAC by cytomorphological features is shown (Table 1).

Table 1: Distribution of liver diagnosis.

Categories	Number of cases	%
Inadequate	0	0
Inflammatory lesions	6	10.5
Benign neoplastic	4	7.1
Suspected malignant neoplastic	0	0
Malignant neoplastic	47	82.4
Total	57	100

Table 2: The distribution of cytodiagnosis of SOL of liver in 57 cases are.

Broad category	Cytological diagnosis	No. of cases		Total cases	Percentage of all cases (%)		
		Male	Female				
Inflammatory/ Non-neoplastic lesion	Nodule of post-hepatic cirrhosis	2	1	3	5.27		
	Pyogenic abscess	2	1	3	5.27		
Benign neoplastic	Hepatic adenoma	0	2	2	3.50		
	Focal nodular hyperplasia	2	0	2	3.50		
Malignant neoplastic	Hepatoblastoma	1	0	1	1.75		
	Primary malignancy (n=18)	Hepato Cellular carcinoma (n=17)	Well differentiated	9	1	10	17.55
			Moderately differentiated	2	1	3	5.27
			Poorly differentiated	3	0	3	5.27
			Fibrolamellar variant	1	0	1	1.75
	Secondary malignancy (n=29)	Adeno carcinoma (n=20)	Well differentiated	7	7	14	24.56
			Moderately differentiated	1	2	3	5.27
			Poorly differentiated	2	1	3	5.27
		Squamous cell carcinoma	1	0	1	1.75	
		Small cell carcinoma	3	0	3	5.27	
		Neuroendocrine tumour	1	0	1	1.75	
		Ductal cell carcinoma	0	2	2	3.50	
		Lymphoma	0	1	1	1.75	
		Adeno-squamous carcinoma	1	0	1	1.75	
	Total	38	19	57	100		

Specific cyto-diagnosis achieved is shown in Table 2. There were 3 cases reported as poorly differentiated adenocarcinoma on FNAC and 3 cases as PDHCC. The features that could differentiate between these 2

overlapping cyto-morphologies by logistic regression of all features taken together ,observed were as follows- Absence of normal or benign hepatocytes in aspirates ,presence of the nuclear inclusions, absence of

reminiscent cell arrangement suggesting adenocarcinoma such as attempted glands, absent background material of mucin but presence of fine lipid vesicles and presence of atypical monstrous stand out bare nuclei. The metastatic cytodiagnosis was offered in 29 cases of which 20 cases (68.96%) were from known primaries and 9 cases (31.04%) were from unknown primary (Table 3). Tissue block diagnosis were available in 46 cases. In 7 cases, the

tissue blocks could not be performed and in 4 cases, tissue blocks were of poor tissue material to entertain the tissue diagnosis. The overall sensitivity was 97.61% and specificity was 100%. The PPV and NPV were 100% and 97.82% respectively when all 46 cases of SOL are considered together for comparison. Overall diagnostic accuracy was 98.85%.

Table 3-Primary sites of metastatic deposits.

Cyto-diagnosis of the metastasis	No. of cases of known primary deposits	Site of primary	No. of cases from primaries	Percentage (%)
Adenocarcinoma	12	Colon	4	20
		Gall bladder	2	10
		Pancreas	1	5
		Stomach	1	5
		Ovary	2	10
		Lung	1	5
		Prostate	1	5
Small cell carcinoma	2	Lung	1	5
		Cervix	1	5
Ductal cell carcinoma	2	Breast	2	10
Squamous cell carcinoma	1	Oesophagus	1	5
Lymphoma	1	Small intestine	1	5
Neuroendocrine tumour	1	Pancreas	1	5
Adeno-squamous	1	Lung	1	5
Total	20		20	100

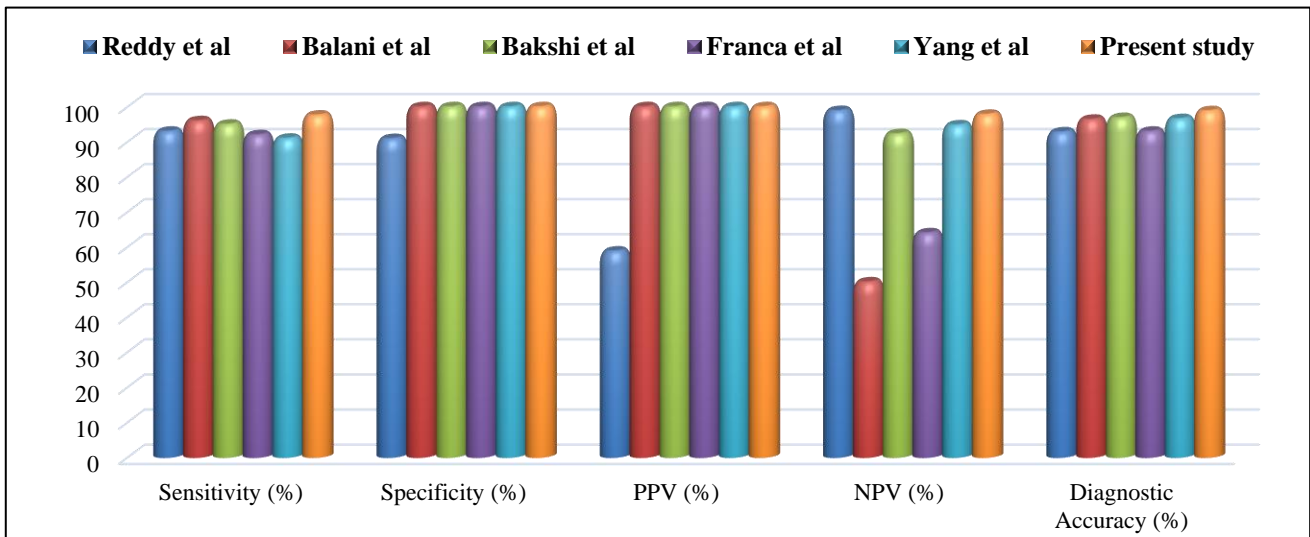


Figure 1: Comparison of statistical parameters between literature and present study.

DISCUSSION

The studies on FNAC of SOL of liver reviewed for the present work, have observed that SOL pathology is

distributed maximally in age group of 51-70 years with pathology either being hepatocellular carcinoma (Primary hepatic malignancy) or metastatic disorder. The present study is in agreement for age group where SOL presents

chiefly beyond 50 years of life with underlying pathology of primary and secondary malignant diseases as reported in the studies of Tailor et al, Yasin et al, Thiruman et al, and Kaur and Selhi.¹⁻⁴ The youngest patient as observed in present study is of 19 years and diagnosed as Hepatoblastoma. Similar observation has been made in the studies of Singh et al, Khanna et al, and Tailor and Kothari.^{1,5,6} Male pre-dominated over female as the study population in the present study is in ratio of 2:1. Similarly, the studies of Ahuja et al, and Kaur and Selhi, reported male predomination in the subjects of SOL from range of 2:1 to 4:1.^{4,7} The highest percentage of clinical diagnosis was of HCC (43.85%) followed by metastatic liver diseases (26.3%). The other studies of Patel et al, and Kumar et al, have the similar clinical diagnosis pattern.^{8,9}

The patients who have been diagnosed with primary HCC in present study didn't show remarkable values for ALT, AST and ALP for a non-neoplastic, neoplastic or malignant neoplastic lesion. Similar observations have been recorded with Ahuja et al. who have concluded that the liver enzymology is not sufficient to characterize the type of disease process within SOL of liver.⁷

The literature reviewed for present study have commented on wide discordance between clinical diagnosis and ultrasonography as necessitating the FNAC of SOL of liver that would result in the mismanagement of sizeable number of patients. The present study recorded 25 discordant diagnosis when clinical diagnosis is compared with that of USG findings which is similar to observation made by Patel et al, and Sudhakar et al.^{8,10}

The utility of cell block of aspirates of SOL of FNAs as paraffin block for histological assessment of SOL has been quoted to be useful and worthy in the studies of Singh et al, Goel et al, Reddy et al, and Sukumaran et al.^{3,5,11,12} The present study made a similar observation about utility of cell block of needle aspirates to achieve tissue diagnosis for comparison with cytodiagnosis.

The cytodiagnosis of SOL in present study had wide spectrum of distribution as shown in Table 2. Almost similar distribution of SOL were quoted in the studies of Tailor and Goel K et al, and Balani et al, with objective similar to present study.^{1,11,19} The commonest cytodiagnosis of SOL over the age of 40 years was of Metastatic liver disease irrespective of gender i.e. 29 cases followed by primary hepatic malignancy i.e. 18 cases (1 Hepatoblastoma, 17 HCC). Similar observations for occurrence and cytodiagnosis for SOL over the age of 40 years are made by Goel et al, with 68% metastatic liver disease and 15% primary hepatic malignancy, Balani et al, who reported 56.5% metastatic lesions followed by primary hepatic malignancy constituting 30.4% cases.^{11,19}

The present study observed 2 cases (3.50%) of Hepatocellular adenoma in females. The diagnosis was

offered as the hepatic adenoma supported by the sonography findings. The observation of Chowdhary et al, about the cytodiagnosis of adenoma was much higher than the one observed in present study.²¹ The particular study reported 11.5% cases as hepatic adenoma in total of 94 cases of hepatic SOL. The study has taken into consideration the USG findings before interpretation of cytomorphology as hepatic adenoma.

The grading of HCC in the present study reported 58.82% as WD i.e. Grade 1 occupying the maximum space of grading followed by MD (17.65%) and PD (17.65%). There was a single case of Fibrolamellar variant (5.88%) of HCC. There is a variability of reporting the grading of HCC in the literature with observation made by present study. The study of Balani et al, reported preponderance of Grade 1 HCC diagnosis which is similar to the observation of present study.¹⁹ However, the other studies of Choudhary et al, Arathi and Giriyan, Mane et al, Goel et al, and Rasania et al, reported maximum number of MD and PD HCC.^{11,18,20,21}

The other diagnostic difficulty by the reporting cytopathologist faced across is the difference between PDHCC and metastatic PD adenocarcinoma. The present study encountered 3 cases of PD adenocarcinoma and 3 cases of PDHCC. By logistic regression, it was observed that the most familial and sensitive feature that would differentiate between above 2 entities by cytomorphology were presence of dual population of benign hepatocytes and malignant epithelial cells, absence of nuclear inclusions, presence of reminiscent cell arrangement of gland formation were the features that suggested PD adenocarcinoma while dissociated hepatoid epithelial cells with presence of nuclear inclusions in the background of lipid vesicles with bare monstrous nuclei and absence of mucin suggested PDHCC. These features at differentiation of 2 overlapping cytomorphologies of above situations have been reported empathetically by the studies of Chhieng et al, Mallikarjuna et al. and Raza et al.^{17,22,23} The studies of Ahuja et al, and Balani et al, reported the differentiation between WDHCC and MDHCC from WD adenocarcinoma with ease on cytomorphological examination.^{7,19}

With the known primary, the diagnosis of SOL as metastatic lesion was observed to be relatively easy as it compared well with the cytomorphology of malignant lesions at its primary sites. The studies reviewed for the present work encountered the situations for metastatic SOL both with known primary and occult primary. These studies opined that the cytodiagnosis of metastasis could be done easily if the malignancy is well differentiated and/or known primary exists. These enables relatively easy to reach at diagnosis which is usually required to suggest prognosis (staging) and treatment outcome. The present study made the similar observation for distinctness of the cytomorphology of differentiated metastatic lesions so also relative ease at its diagnosis as

quoted by Valecha et al, Thiruman et al, Goel et al, Khanna et al, and Gupta et al.^{3,6,11,13,15}

The present study observed to have overall values of Specificity (100%), Sensitivity (97.61%), NPV (97.82%), PPV (100%) and Diagnostic accuracy (98.85%). The studies of Yang et al, and Bakshi et al, have mentioned high values of above parameters over 90% (except NPV) for FNAC for the diagnosis of SOL of liver which is comparable with observations of present study (Figure 1).^{24,25}

The studies of Saem et al, brought out a sensitivity of 66.7% and specificity of 85.18% for FNAC in diagnosis of SOL of liver which is unparallel or incomparable with results in present study.²⁶ So, also Kumar et al, reported sensitivity of 78.26% and Kuo et al, who reported sensitivity of 78.4% which is low as compared to other published studies and present study.^{9,27} There are reports of Balani et al, and Franca et al, who have reported low NPV of 50% and 64% respectively which is a contrary observation to the studies reviewed for literature as well as present study which has quoted NPV of 97.82% because of low false negative reporting in present as well as above quoted studies.¹⁹ A report of Reddy et al, has reported low PPV of 58.8%.¹² These values are contrary to the reported studies reviewed for present work. The studies of Balani et al, Bakshi et al, and Franca et al, has reported PPV of 100% due to low false positive cases.^{19,25} The present study made the similar observation as studies of Balani et al, Bakshi et al, and Franca et al, but contrary to the report of Reddy et al.^{12,19,25} Of the 46 cell blocks study of needle aspirates, there was a single false negative cytodiagnosis of FNAC of SOL of liver in present study where cell block revealed metastatic deposits of adenocarcinoma. This was due to a non-representative cell yield in the smears of FNAC and cell masking partly by blood. Such limitation for erroneous diagnosis of false negative has been quoted in the studies of Roy et al, Nazir et al, and Sudhakar et al.^{10,28,29}

CONCLUSION

FNAC is concluded to be first rank diagnostic procedure in diagnosis of SOL of liver of varied etiology with high values of NPV and PPV. It is also concluded that it helps in staging of metastatic malignancies.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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