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Original Research

Cytopathological Evaluation Of Image Guided Fine Needle Aspiration Cytology Of Liver Lesion With Cell Block Correlation.

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Abstract:

Background and rationale: Image guided fine needle aspiration cytology of liver lesion is a widely used procedure, providing rapid and accurate diagnosis. However, exact diagnosis is not possible always with information obtained by fine needle aspiration (FNA) cytological material and the possibility of indeterminate diagnosis exists. Therefore, we attempted to obtain additional information via the preparation of Cell block (CB) from the residual material of aspirates to aid in increasing the diagnostic accuracy.

Objectives:

1. To study cyto-morphological features in various hepatic lesions
2. To assess utility of cellblock preparation method in increasing sensitivity of cyto-diagnosis in liver lesion.

Study Design: Descriptive study

Methods and Material: We conducted a descriptive study at a tertiary care teaching hospital(Karnataka Institute of Medical Sciences, Hubli) for a period of one and half years which included a total of 75 cases with suspected liver mass. Using image guidance, fine needle aspiration was done and smears were prepared. The rest of the material present in the needle hub was submitted for cell block preparation.

Statistical analysis used: The quantitative variables were summarised using mean and qualitative variables as proportions. Sensitivity, specificity and positive predictive value were calculated for Image guided FNAC with Cell block as gold standard.

Results: Most common lesion was hepatocellular carcinoma. Sensitivity, specificity and diagnostic accuracy of smear preparation were 98%,90.2% and 96.6% respectively. With FNA and CB together, the sensitivity and specificity increased to 100%.

Conclusions: In this study, Image guided FNAC is found to be highly sensitive and accurate in diagnosing the hepatic lesions. Conventional smears can be adequate as a diagnostic tool in routine practices, but adding the cell block as an adjunct method will help us to increase the diagnostic accuracy and sensitivity especially in difficult to diagnose cases.

Key-words: Image guided FNAC, Liver lesions, Cell block

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Introduction:
The liver is one of the common organs for various non-neoplastic and neoplastic lesions. These include primary liver tumours, secondary deposits, cysts, abscesses and granulomas.
Image guided fine needle aspiration cytology (FNAC) is widely used, rapid, cost effective and minimally invasive method for the diagnosis of the liver lesions[1,2]. In addition to conventional smears, an attempt to obtain additional information can be made via the preparation of cell block from residual material remaining after completion of cytology preparation. This material often contains tissue fragments, which can give valuable information that cannot be processed by cytology[1,3]. Cytopathological evaluation and cell block preparation from FNA together yield two differing, complementary view of the same cell population[2].
The present study is undertaken to evaluate the cyto-morphological features of various hepatic lesions in detail as well as to assess the utility of cell blockpreparation method in increasing the diagnostic accuracy.

Methodology:

Primary objective: To study cyto-morphological features in various hepatic lesions.

Secondary objective: To assess utility of cellblock preparation method in increasing sensitivity of cyto-diagnosis in liver lesion.

Study Design: This is a descriptive study carried out in the cytology division of a tertiary care teaching hospital.

Study setting: Pathology Department of Karnataka Institute of Medical Sciences, Hubli, Karnataka.

Study duration: One and half years from October 2017 to March 2019 after obtaining the Ethical clearance from the Institutional Ethical Committee.

Study Subjects: Patients presenting with radiologically detected liver mass lesions were included in the study.

Exclusion Criteria: All abdominal mass cases other than liver lesion, patient with any bleeding disorder, non-co-operative patients and hydatid cyst diagnosed on ultrasound were excluded from this study.

Ethical considerations: Approval was obtained from institutional ethical committee prior to conducting the study(No.KIMS/PGS/SYN/447/2017-18).Informed written consent was obtained from each patient.

Method of data collection:
The patients were selected regardless of their age, sex, socio-economic status, and occupation. A detailed clinical history was taken and radiological findings were noted. Required tests like bleeding time, clotting time, prothrombin time and activated partial thromboplastin time were done. Under image guided techniques including Ultra-sonography (USG) and Computed tomography (CT), FNAC was done using lumbar puncture needle of 22 gauge. Minimum of 4 smears were prepared which included wet alcohol fixed smears for Haematoxylin & Eosin (H&E) and Papanicolaou's (PAP) stain, and air-dried smears for May-Grunwald Giemsa (MGG) stain. The rest of the material present in the needle hub and syringewere submitted in cell block solution (mixture of equal quantity of 100%

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ethanol and 10% formalin) using needle rinse method. Centrifugation of the sample was done at 2000 rpm for 2-3 minutes. Cell button submitted for routine histopathological examination. Paraffin embedded;4-6-micron thick sections will be routinely stained with H&E stain. Whenever necessary, histochemical special stains and Immunohistochemistry (IHC)were used.

Statistical analysis:
The data obtained was analysed by SPSS 21.0 software trial version. Microsoft word and excel was used to assimilate the data and prepare the article. The quantitative variables were summarised using mean and qualitative variables as proportions.

Results:
A total of 75 cases with liver lesion, clinically or radiologically diagnosed, were investigated. Our study showed a wide range of age distribution ranging from 22-85 years. The mean age of presentation was 58.9 years. Incidence of non-neoplastic lesion were predominantly seen in 5th and 6th decades and neoplastic lesions were more common in 6th and 7th decades. Out of the total 75 cases, 69.3% were males and 30.6% were females.
Majority of the patient presented with complaints of pain abdomen (74.67%). Most common imaging technique used was USG (95%) and in the remaining cases samples were obtained using CT guidance.
Out of 75 cases, adequate material was obtained on FNAC in 70 cases (93.3%). Adequatematerial for cell block was obtained in 60 cases (80%).
Out of the 70 cases with adequate material, 56 were neoplastic and 14 were non neoplastic cases.
Non neoplastic lesions included diffuse parenchymal liver disease, pyogenic abscess, regenerative nodule and simple cyst. Among these 56 neoplastic lesions, 35 were Hepatocellular carcinoma (HCC) and 20 cases were metastatic deposits and the remaining 1 case was diagnosed as haemangioma [Table 1].

Table 1.Type of lesions.

Lesions	Diagnosis	Frequency
Non neoplastic	Diffuse parenchymal liver disease	07(10%)
	pyogenic abscesses	04(5.7%)
	Regenerative nodule	01(1.42%)
	Simple cyst	02(2.85%)
Neoplastic	Hepatocellular carcinoma	35(50%)
	Metastatic deposits	20(28.5%)
	Haemangioma	01(1.42%)

NON NEOPLASTIC LESIONS
Most common non neoplastic lesion was diffuse parenchymal liver disease. The predominant pattern of arrangement of hepatocyte in these caseswere clusters and sheets. All the cases showed intra cytoplasmic bile pigment and presence of bile duct epithelium. Out of the 7 cases,5 cases showed fatty change. Cell block section showed sheets and groups of benign hepatocytes having abundant cytoplasm, central round nucleus with fine granular chromatin and prominent nucleoli. Micro vesicular and macro vesicular fatty changes were seen in the 5 cases.Special stain Masson trichrome stain was used in a case of diffuse parenchymal lesion presented with history of cirrhosis and it demonstrated blue stained collagen amidst the hepatocytes. Smears of pyogenic abscesses showed predominantly sheets of neutrophils and nuclear debris in a necrotic background. Clusters of benign hepatocytes were present. Cell block sections showed sheets of inflammatory cells and groups of hepatocytes.

PRIMARY NEOPLASTIC LESIONS

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Hepatocellular carcinoma was the most encountered lesion of the liver in the present study (50%).Detailed cyto-morphological analysis of each case was done and compared with cell block findings[Table 2].
Among 35 cases, aspiration was highly cellular in 26 cases and was moderate in remaining 9 cases. Out of the 35 cases, 20 cases showed classic trabecular pattern. It was the commonest pattern seen in well differentiated HCC. Other patterns commonly encountered were clusters and dispersed single cell arrangement. Clusters of hepatocytes with traversing blood vessels were seen in 30 cases (85.7%). Endothelial wrapping around the trabecula were seen in 15 cases (42.8%). Pleomorphic nuclei were observed in 88.5% of the cases. All the cases showed high nuclear cytoplasmic ratio, prominent macro nucleoli and atypical naked nuclei(100%). Intra nuclear cytoplasmic inclusion was present in 23 cases (65.7%). Presence of intracytoplasmic bile pigment could be appreciated in 8 cases (22.8%) and bile duct epithelium was absent in all the 35 cases.

Table 2. Cytomorphological analysis

Cytological feature	Number of cases
High cellularity	26 (74.3%)
Trabecular pattern	20 (57%)
Traversing blood vessels	30 (85.7%)
Endothelial wrapping	15 (42.8%)
High N/C ratio	35 (100%)
Pleomorphism	31 (88.5%)
Coarse chromatin	35 (100%)
Prominent nucleoli	35 (100%)
Naked nuclei	35 (100%)
Intra nuclear inclusion	23 (65.7%)
Intra cytoplasmic bile	08 (22.8%)
Absent bile duct epithelium	35 (100%)

Hepatocellular carcinoma cases were further sub classified depending on these cytological and nuclear features. Out of these 35 cases, 8 were well differentiated HCC [Figure 1], 24 were moderately differentiated HCC and 3 cases were poorly differentiated HCC.

The well differentiated HCC showed high cellularity in majority of the cases with tumour cells arranged predominantly in trabecular pattern (75% of well differentiated HCC). These tumour cells were showing absent or mild pleomorphism with abundant granular cytoplasm, central round nuclei with coarse chromatin, prominent nucleoli and slightly increased nuclear cytoplasmic ratio. Endothelial wrapping was present in the 7 cases of well differentiated HCC (88%). Traversing blood vessels was present in 6 cases of well differentiated HCC (75%). The predominant pattern identified in cell block sections were trabecular pattern. Thickness of trabeculae was more than 3-4 cell thickness in all the cases.
The moderately differentiated HCC showed cellular smears with tumour cells arranged predominantly in clusters and in trabecular pattern. These tumour cells were moderately pleomorphic with high N/C ratio, round to oval nucleus with coarse granular chromatin, multiple macro nucleoli and moderate cytoplasm. Traversing blood vessels was present in 22 cases of moderately differentiated HCC (92%). Endothelial wrapping was present in the 8 cases of moderately differentiated HCC (33.3%). Cell block showed tumour cells arranged in small groups and trabecular pattern.
The cases of poorly differentiated HCC showed discohesive tumour cells arranged in dispersed singles. These tumour cells were having scant cytoplasm, marked nuclear pleomorphism and multiple macro nucleoli. Multi nucleated tumour giant cells were frequently observed. Cellblock sections showed dispersed tumour cells in singles and small groups.

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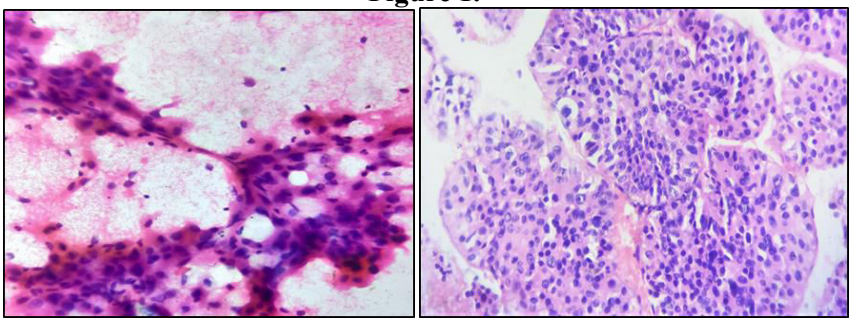
Out of 35 cases, 20 cases were confirmed with IHC using marker HepPar 1. These cases showed diffuse granular cytoplasmic positivity.

Figure 1.

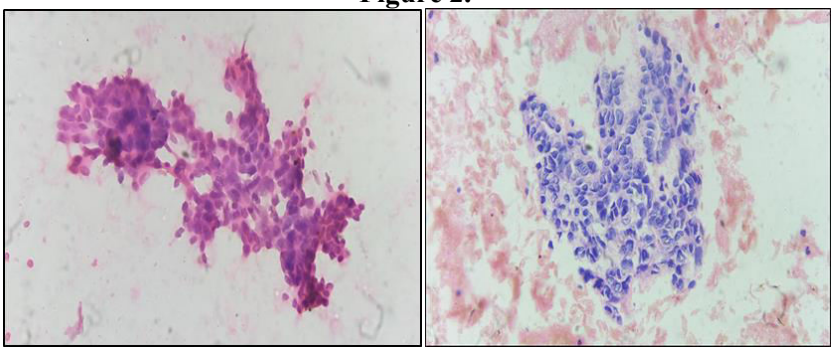


Well differentiated HCC (FNAC- H&E,40X,CB-H&E,20X)

METASTATICDEPOSITS

The remaining 20 cases out of the 56 neoplastic lesionswere reported as metastatic deposits to the liver. Of these, 18 cases were metastatic adenocarcinoma deposits [Figure 2], 1 case was metastaticadeno-squamous carcinoma deposit and the remaining 1 case was malignant melanoma deposit. Most common primary site of malignancy in this study was Colon (6 cases) followed by pancreas (4 cases), oesophagus (3 cases), breast (2 cases), ovary (1 case) and lung (1 case).

Figure 2.



Metastatic deposits from Papillary Carcinoma Ovary (FNAC-H&E,40x & CB-H&E,40x)

Most common pattern of arrangement of tumour cells observed in these metastatic deposits were clusters followed by acinar pattern and papillary pattern.

The present study predominantly comprised of metastatic adenocarcinoma deposits (90% of metastatic deposits). In adenocarcinoma deposits, tumour cells were cuboidal to columnar with hyperchromatic nuclei and moderate amount of vacuolated cytoplasm. Deposits from colon carcinoma showed intracellular and extra cellular mucin in the background. Cell block sections showed tumour cells arranged in clusters, acini and papillary pattern and having hyperchromatic nuclei and vacuolated cytoplasm. Special stain with PAS showed intracytoplasmic positivity.

Deposit from malignant melanoma showed large tumour cells with irregular nucleus, prominent nucleoli and moderate cytoplasm containing melanin pigments. Cell block sections showed sheets of tumour cells with intracellular and extra cellular melanin pigments. These cells showed strong cytoplasmic positivity with HMB-45 in immunohistochemistry [Fig 3]

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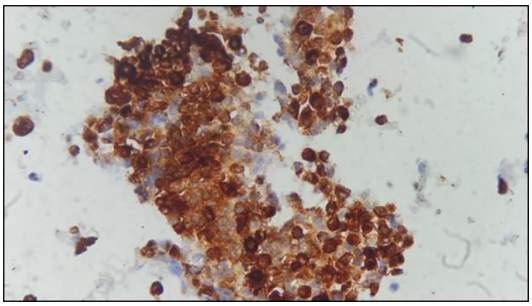
FIGURE 3



IHC on cell block section showing intracytoplasmic positivity for HMB-45 in malignant melanoma

Out of the 20 cases, 3 cases were of unknown primary. Clinical details of these cases werecollected and an attempt was made to predict the primary site of tumour by assessing the cyto morphology in smears, histo-morphology and IHC in cell block [Table 3]

Table 3.Cytomorphology in smears, histomorphology and IHC in cell block a comparison

CLINICAL DIAGNOSIS	FNAC DIAGNOSIS	CB DIAGNOSIS	IHC
CT; pancreatic solid lesion	Metastatic adenocarcinoma deposits	Adeno squamous deposits	CK5 +, CK7+
Suspected case of primary Carcinoma stomach	Metastatic adenocarcinoma deposits	Metastatic adenocarcinoma deposits	CK7+
Metastatic adenocarcinoma deposits	Metastatic adenocarcinoma deposits	Cholangiocarcinoma	Inadequate

ANALYSIS OF EFFICIENCY OF SMEARS

Diagnostic sensitivity, specificity and positive predictive value of FNAC smears were calculated considering cell block as gold standard[Table 4].

Table 4. Diagnostic test evaluation parameters of FNAC

Diagnostic test parameter	Value
Sensitivity	98%
Specificity	90.2%
Positive predictive value	96.6%

Table 5.Comparison of cytological features of HCC in different studies

Cytological features	Shashikala V et al[12]	Cohen et al[19]	**Present study**
High cellularity	-	83%	**74.3%**
Trabecular pattern	50%	65%	**57%**
High N/C ratio	-	71%	**100%**

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Naked nuclei	65%	73%	**100%**
Prominent nucleoli	100%	54%	**100%**
Intra nuclear inclusion	45%	-	**65.7%**
Intra cytoplasmic bile	15%	-	**22.8%**
Coarse chromatin	-	33%	**100%**
Pleomorphism	-	71%	**88.5%**
Absence of bile duct epithelium			**100%**

In 7 cases (10.3%) with difficult or doubtful diagnosis in smears, CB helped to arrive a definitive diagnosis. Difficulties faced in the present study was differentiating non neoplastic lesions from well differentiated HCC, differentiating poorly differentiated HCC from metastatic deposits and detection of the origin of metastatic deposits.Whichever cases had difficulties in differentiating the well differentiated HCC and non-neoplastic liver diseases using smears, we used cell block to confirm the diagnosis. Solid pattern and trabeculae of hepatocytes thicker than 4 cells rimmed by endothelial cells were considered as a feature of HCC.Differentiating poorly differentiated HCC from poorly differentiated metastatic tumour is challenging. IHC using Hep Par-1 antibody was performed in doubtful cases to confirm the diagnosis.
With FNA and CB together, we were able to provide a definitive cyto-pathological diagnosis in all the 60 cases, increasing the sensitivity to 100%.

Discussion:
Fine needle aspiration cytology of the liver was established by Sodenstorm in 1966 with the examination of 500 cases[4].Later in 1976, Haaga et al introduced a method of preciselocalization of lesion by Computed tomography (CT). This allowed accurate positioning of needle in small and deep lesions[5].Over the last 15 to 20 years, FNAC under image guidance has attained an increasing acceptance as the diagnostic procedure of choice for single or multiple focal hepatic lesion.
The use of cell block for processing cytology fluids has been first reported in 1895 by Bahrenberg[6,7]. In addition to the conventional smears, cell block technique has a significant role in diagnostic cytopathology and it is a valuable adjunct towards a more accurate cytological diagnosis complementary to smears and histology[6,7,8]. Cell block preparation are used routinely for body fluids, fine needle aspirations and other cytological samples.
Computed tomography(CT) and ultrasound guidance (USG) are the two main image guidance system using for liver FNAC. In this present study, ultrasonography was the main guidance technique we have used.
In the present study, the mean age at presentation was 58.9 years ranging from 22-85 years which is comparable with the study done by Haqsheefa et al[10] in which the mean age of presentation was 58.8 years. Maximum number of cases were seen between 51 and 70 years of age (6th and 7th decade) (70%) and this is comparable with the study done by Mathew and Nair[11] in 2017 and Shashikala V et al.[12] in 2016. Our study showed a male predominance with male to female ratio of 2.3:1 and these results are similar to the studies done by Haqsheefa et al[10] and Shashikala V et al[12].
Most important requirement for cyto-diagnosis is to obtain a representative sample[13]. In the present study, satisfactory aspirate for cyto-diagnosis on FNAC was obtained in 70 cases (93.3%). This is comparable to 91.67% adequacy obtained in a study done by Rajesh Chandan et al[14]and 96.96% adequacy obtained in the study done by Shashikala Vinayakamurthy et al[12].Adequate material for cell block was obtained in 60 cases (80%) and this is comparable to the study by Nathan et al.[15] who obtained adequate material on cell block in 73.3% cases. Major factor contributing to the optimal preparation of cell block is adequate rinses from fine needle aspiration syringe to extract the residual tissues.
Out of the 70 cases studied, majority of the lesions were neoplastic (80%) and the remaining 20% were non neoplastic lesions. This is similar to the distribution of cases found in the study by Balani et al.[13](2013), study by Rajesh Chandan et al.[14](2018) and study by Shashikala Vinayakamurthy et al.[12].

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Most common non-neoplastic lesion identified in the current study was diffuse parenchymal liver diseases (10%). This is similar to the study conducted by Rajesh Chandan et al.[14]and the study conducted by Asghar F and Riaz S[16].In an another study conducted in Bangalore, Karnataka by Shashikala V et al[12], most common non-neoplastic lesion identified was pyogenic abscess.

The present study showed 50% of Hepatocellular carcinoma, 28.5% of metastatic carcinoma and 1.4% of haemangioma. Hence, the most common lesion of the liver in our study was hepatocellular carcinoma (HCC). This is comparable with different studies done by Shashikala Vinyakamurthy et al[12]., Mohammed AA et al.[1] and Sumana BS[3] in which the majority of cases were HCC. However, our study differs from the other studies done by Khanna et al.[17], Rajesh Chandan et al.[14] and Nosher et al.[18] since the majority of cases in their studies were metastatic deposits.

HCC is the most common primary malignancy of the liver and early diagnosis of HCC is important because of prognostic implication[10].Cohen et al.[19], proposed three primary useful criteria to discriminate between HCC and non- neoplastic liver lesion in FNAC. These features were increased nuclear cytoplasmic ratio (N/C), trabecular pattern and atypical naked nuclei.In the current study, we examined 10 cytological features that have been reported as useful in the literature for diagnosis of HCC. We analysed the utility of these features in all the 70 cases of liver FNAC. The current study identified the following features in all the cases of HCC; atypical naked nuclei in the background, presence of macro nucleoli, absence of bile duct epithelium, high N/C ratio and coarse clumped chromatin. These were the main features used to differentiate between HCC and non-neoplastic liver diseases in the present study. In addition to that, features like high cellularity and trabecular pattern are also considered. Useful features we found in the present study to differentiate between HCC and metastatic deposits are trabecular pattern, atypical naked nuclei, presence of macro nucleoli, intra nuclear inclusion and intra cytoplasmic bile pigment.

Based on above mentioned cytological features, all the cases of HCC were further sub-classified into 3 grades; Well differentiated HCC (8 cases), moderately differentiated HCC (24 cases) and poorly differentiated HCC (3 cases). This is comparable with the study conducted by Haq Sheefa[10] where majority of the HCC cases were moderately differentiated.However, one study conducted in Bangalore, Karnataka by Shashikala V et al[12] showed predominance of well differentiated HCC. Another study conducted in Bhopal by Balani et al[13] showed predominance of poorly differentiated HCC.

In the present study, metastatic deposits to the liver were accounting for 28.5% and major bulk of metastatic tumours in the present study comprised of metastatic adenocarcinoma deposits in 90% of metastatic deposits.This is comparable with the study done by Shashikala V et al.[12]and another study done in Bhopal, Madhya Pradesh by Balani et al[13], 89.6% of the metastatic deposits were adenocarcinoma deposits.The most common primary site of malignancy identified in the present study was colon and pancreas. This is comparable with the studies done by K.Ceyhan et al.[2] and Balani et al.[13]

The distinction between a primary carcinoma liver and metastatic deposits is very important since it has both therapeutic and prognostic significance.The cytological criteria to differentiate HCC from metastatic tumours stated by Bottles et al.[20] include polygonal cells with centrally placed nuclei, malignant cells separated by sinusoidal capillaries, presence of bile, intra-nuclear cytoplasmic inclusions and endothelial rimming.The salient features separating HCC from metastatic adenocarcinoma deposits described by Greene et al[22] were tumour cells in HCC are polygonal or polyhedral, have abundant eosinophilic and granular cytoplasm, macro nucleoli and trabecular arrangement.Whereas tumour cells in metastatic adenocarcinoma deposits are columnar or cuboidal, predominantly have acinar or glandular arrangement, show mucin secretions and inflammatory background.The salient features separating HCC from metastatic adenocarcinoma deposits identified in the current study were also the same.

Deposit from Metastatic melanoma may mimic HCC since tumour cells have several similar features. Also, melanin pigment can resemble various liver cell pigments. History of primary lesion elsewhere and features of melanoma like single cells with eccentric nucleus, single prominent nucleoli, bi-

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nucleation may help distinguish it from HCC. Immunohistochemistry can be done to confirm the diagnosis.

Various studies have reported sensitivity varying from 62-100% and specificity 63% to 100%[1,2,12,13]. In the present study FNAC was able to diagnose the liver lesion with an overall sensitivity of 98% and specificity of 90.2%. The overall accuracy of procedure in the present study was 96.6% which was comparable to the rate of accuracy reported in the studies done by Mohmmed AA et al[1] and Shashikala V et al[12].

The utility of cell block along with routine smears has been evaluated by various authors. In the present study, cell block provided additional information in 10.3% of the cases. In a study done by Liu et al[21], cell block section provided additional information in 12% of the cases andanother study by Nathan et al.[15], described 15.2% of improvement in diagnosis when both smears and cellblocks were studied together.In another study conducted in Bangalore, Karnataka by BS Sumana and Bharathi Muniyappa[3], cell block helped to improve the diagnosis in 15.55% of the cases.

With smear and cell block together, 100% correct diagnosis was achieved in all the 60 cases increasing the sensitivity and specificity to 100%. This is comparable with the study done by Shashikala V et al.[12] The result of this study revealed that diagnostic sensitivity can be increased by adding cellblock as adjunct method along with conventional smears.

Cell block sections displayed cytomorphology clearly recognizable with limited shrinkage. The cyto-morphological features were properly maintained with clear recognition of nuclear and cytoplasmic features. This is corresponding to study done by Kung et al.[23] regarding the staining results on CB where cell block sections showed excellent staining results with IHC.In addition to the role in increasing diagnostic sensitivity, cell block section can also be used for special stains and immunohistochemistry. In the current study, cell block sections showed excellent staining pattern with IHC markers.

Conclusions:

In this study, Image guided FNAC is found to be highly sensitive and accurate in diagnosing the hepatic lesions. Conventional smears can be adequate as a diagnostic tool in routine practice, but adding the cell block as an adjunct method will help us to increase the diagnostic accuracy and sensitivity especially in difficult cases.

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