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CYTOMORPHOLOGICAL CLASSIFICATION OF SPACE OCCUPYING LESIONS OF LIVER AT A TERTIARY CARE CENTER

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ABSTRACT
Single or multiple lesions in liver which are diagnosed by clinicians with patient's history and clinical examination and by radiologist with ultrasonography or Computed tomography scan constitute the main indication for Fine needle aspiration cytology (FNAC) of liver. Since it's an invasive procedure, it's a big challenge even to an experienced cytopathologist. Mainly the problem occurs when a well differentiated hepatocellular carcinoma is mistaken for benign tumor or a poorly differentiated hepatocellular carcinoma is mistaken for a metastatic lesion. Most of these problems could be resolved with the aid of new techniques like immunohistochemistry,molecular studies etc. The problem still persists when these facilities are unavailable.The present study was undertaken to classify the Space Occupying Lesions (SOL) of liver cytomorphologically with emphasis on hepatocellular carcinoma. Fine needle aspiration cytology samples were collected for the duration of one year at a tertiary care center. Each case is evaluated according to history, chief complaints and clinical diagnosis. Inclusion criteria for our study are patients with history of liver disease, who had clinically and radiologically diagnosed liver disease, both males and females and any age group. Exclusion criteria- those who are clinically diagnosed but radiologically don't have any space occupying lesion in liver. With all aseptic precautions and under the guidance of ultrasound, FNAC was done after taking consent. Total numbers of cytology samples were 3180 which included hepatic and non-hepatic lesions.The numbers of liver Space occupying lesions (SOL) samples were 52 that accounts to 1.64 % of the total number of liver samples. Male to female ratio was 5.5:1(43 males, 9 females) and age ranges from 16 months to 86 years. The samples were divided into non-neoplastic (9.62%), neoplastic (76.92%) and inadequate for opinion (13.46%). Acute inflammation (Abscess)(1.9%), chronic nonspecific inflammation(3.8%), and regenerating nodules(3.8%) were the non-neoplastic lesions encountered. Neoplastic lesions were more common than non-neoplastic lesions.Neoplastic conditions were classified into primary and secondary. Primary neoplasms were further classified into benign and malignant. The most common primary malignant tumor was hepatocellular carcinoma (53.8%). Second most common SOL of liver is secondaries (19.2%). Other malignant tumor seen was hepatoblastoma (3.8%). Secondaries were deposits of colorectal adenocarcinoma.The sensitivity, specificity and diagnostic accuracy of FNAC in our study was 86.5%, 94.3% and 100% respectively. FNAC is an excellent procedure to diagnose Space Occupying lesions of liver. It can be taken from both right and left lobes and from superficial or deep lesions.

KEYWORDS: Fine needle aspiration cytology, Space Occupying Lesions of liver, abscess, regenerating nodule, Hepatocellular carcinoma and secondaries.

INTRODUCTION
Lesions which are single or multiple and demonstrated by clinical history, palpation, ultrasonography, and or computed tomography scan constitute the main indication for Fine needle aspiration cytology (FNAC) of liver. In this method a fine needle usually 21G is inserted into the liver mass under ultrasound or computed tomography scan guidance. Once the needle is inside the lesion it is moved forward and backward twice or thrice in all directions, after this material is aspirated which comes in syringe. This material is transferred on the slide and a smear is prepared with the aid of another clean slide. This smear is fixed and stained with any cytological stains. We used Hematoxylin and eosin to stain all the smears. Since it's an invasive procedure, it's a big challenge even to an experienced cytopathologist. The challenges which are usually faced by cytopathologist are 1. FNAC procedure, whether to use fine needle or trucut biopsy needle 2 Type of stain to be used 3. Accurate diagnosis since each lesion has a

different type of management. In the present study we use 21G fine needle to aspirate the material. The major advantage with this is the minimal complications and easiness of procedure. The only complication which we encountered is the pain, otherwise bleeding and needle tract metastasis is reported by other methods. There is no bleeding with this procedure. So this can be used in patients with bleeding disorders also which is very common in liver diseases.[5] Both under diagnosis and over diagnosis is dangerous. If a malignant lesion is underdiagnosed as adenoma, it's a problem and if a benign lesion is diagnosed as carcinoma, then this also is troublesome. Correct diagnosis of liver space occupying lesions is very important since the management depends mainly on diagnosis.[21] Newer methods like immunohistochemistry, Insitu hybridization, and molecular studies are very helpful in arriving at a specific diagnosis. To differentiate these lesions first we have to see whether it's a primary tumor or metastatic deposit. In primary we have to differentiate hepatocellular carcinoma with other benign tumors like adenoma and non-neoplastic lesions like abscess, regenerating nodules, cysts and granulomas.

Ultrasonography and CT scan guided fine needle aspiration cytology is very helpful even in focal liver diseases.[22]Problem occurs when a well differentiated hepatocellular carcinoma is mistaken for benign tumor. The features of well differentiated carcinoma which usually mimic adenoma are polygonal cells with moderate amount of cytoplasm which is seen in both adenoma and carcinoma but the feature which distinguish them is the high N: C ratio in HCC.[20]anda poorly differentiated hepatocellular carcinoma is mistaken for a metastatic lesion.[9], [20] Most of these problems could be resolved with the aid of newer techniques like immunohistochemistry, molecular studies etc. The problem still persists when these facilities are unavailable.

We undertook the present study firstly to know the Cytomorphological patterns of liver space occupying lesions and to classify SOL of liver with emphasis on hepatocellular carcinoma based on cytomorphology.[14, 15] Hepatocellular carcinoma is graded into well differentiated, moderately differentiated and poorly differentiated based on cytological criteria which is discussed in detail.[13]

MATERIALS AND METHODS

The present study was done on the samples collected from Osmania General Hospital, Hyderabad, India. It's an invasive procedure, and consent was taken for the procedure from patients. During the study period each patient who has complaints of any liver disease is evaluated according to history, chief complains and clinical diagnosis. An ultrasonography is done for these patients and on diagnosis with mass lesion in liver, fine needle aspiration cytology was performed.

The total cytology specimens were 3180.We received 52 cases of liver space occupying lesions in this one year period.So the percentage of liver SOL is 1.64%. Males are affected frequently with Male to female ratio of 5.5:1. There is a wide range of ages with youngest being 16 months and oldest 86 years.

We use 21 G fine needle to aspirate these lesions. Once the needle is inside the lesion the material is aspirated with forward and backward movements in all directions. Materials obtained is transferred onto clean glass slide and spread with another slide to make a thin smear. This smear is fixed in a fixative and later stained with Hematoxylin and eosin and studied under light microscope. Two cytopathologist separately studied all the smears and gave their opinion. There is no difference of opinion between the two in any case.

We divided our samples into three categories: 1. Non-neoplastic 2. Neoplasticand 3. Inadequate for opinion. Both neoplastic and non-neoplastic lesions were common in males. Inadequate for opinion means some smears are diagnosed as mass lesions on ultrasound but during FNAC procedure only blood and blood elements was aspirated without any hepatocytes or atypical cells.

RESULTS

Out of 52 cases, there were 43 males and 9 females. Age wise distribution of cases is given in Table 1

Table 1: Age wise distribution of Space Occupying lesions of Liver.

AGE (in years)	Number of Cases		
	Neoplastic lesions	Non-neoplastic lesions	Inadequate for opinion
01 – 10	02	---	---
11 – 20	---	---	---
21 – 30	---	01	02
31 – 40	10	02	03
41 – 50	13	02	02
51 – 60	07	---	---
61 – 70	04	---	---
71 – 80	03	---	---
81 – 90	01	---	---
TOTAL	**40**	**05**	**07**

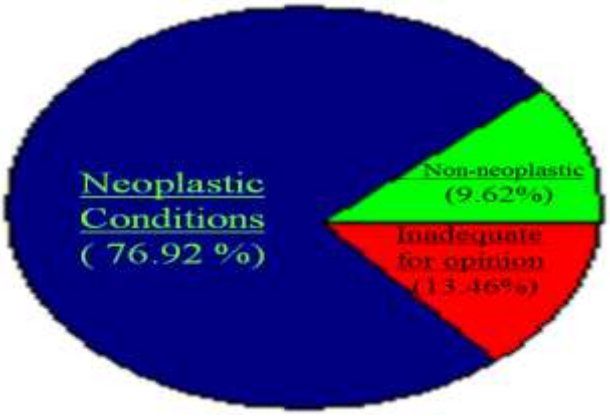


Diagram 1: Distribution of SOLs of liver.

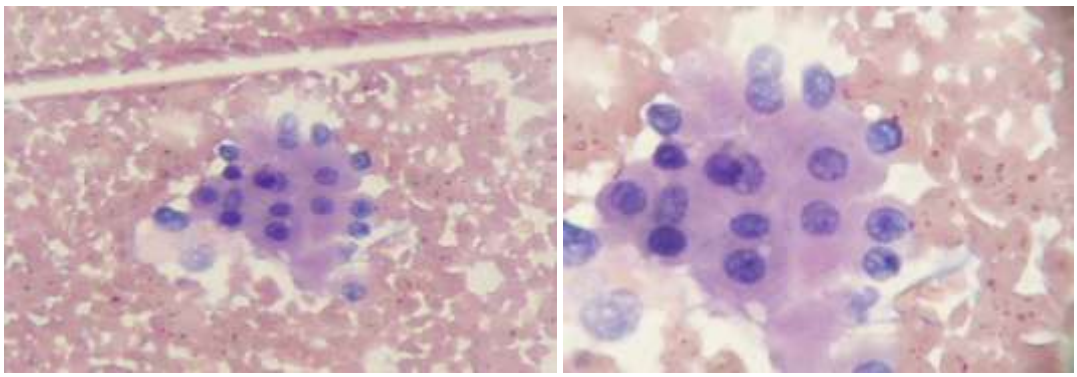
5 cases (9.62%) were non-neoplastic, 40 cases (76.92%) were neoplastic and the remaining 7 cases (13.46%) were inadequate for opinion. 40 neoplastic conditions were divided into primary and secondary. Primary is further divided into benign and malignant. Table 2 shows Cytomorphological patterns and classification of SOLs along with percentage of these tumors.

Table 2: Cytomorphological Patterns of Space Occupying lesions of liver.

Diagnosis	Number of cases	Percent
Non-neoplastic Lesions	**5**	**9.62**
Abscess	1	1.92
Chronic nonspecific inflammation	2	3.85
Regenerating nodules	2	3.85
Neoplastic Lesions	**40**	**76.92**
Hepatoblastoma	02	3.85
Hepatocellular carcinoma	28	53.84
Secondaries	10	19.23
Inadequate for Opinion	**7**	**13.46**
TOTAL	**52**	**100**

The Cytomorphological spectrum for the non-neoplastic Lesions are: 1. Abscess- one case (1.9%) 2. Chronic inflammation-two cases (3.8%) and 3. Regenerating nodules-two cases (3.8%). Cytosmear of abscess showed abundant neutrophilic infiltration and for chronic nonspecific inflammation, the cytosmear showed neutrophilicand lymphocytic infiltration against necrotic background. In regenerating nodules, the cytosmear showed clusters of hepatocyteswith several binucleate hepatocytes.

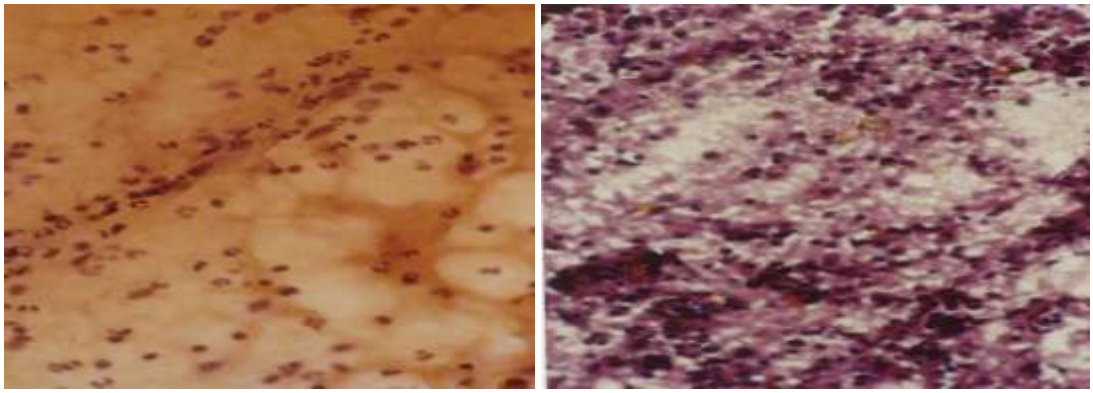
CYTOSMEAR OF NORMAL HEPATOCYTES



A **B**

Figure 1:FNAC of Liver: Normal Hepatocytes-Round to polygonal cells arranged in cohesive sheets with indistinct cell borders, abundant granular cytoplasm, few cells shows vacuolation. (H&E,A x100, B x400)

ABSCESS AND CHRONIC NONSPECIFIC INFLAMMATION



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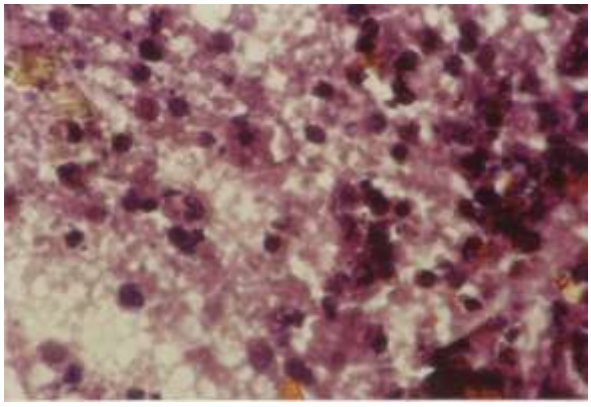


C

Figure 2: FNAC of Liver: A – Abscess-Cytosmear shows abundant neutrophilic infiltration.B&C – Chronic Inflammation- Cytosmear shows abundant lymphocytic infiltration along with neutrophils against necrotic background.(H&E, A x 100, B x100, C x400).

REGENERATING NODULE

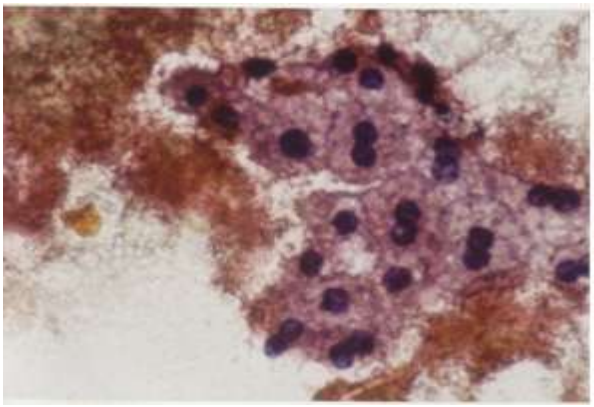


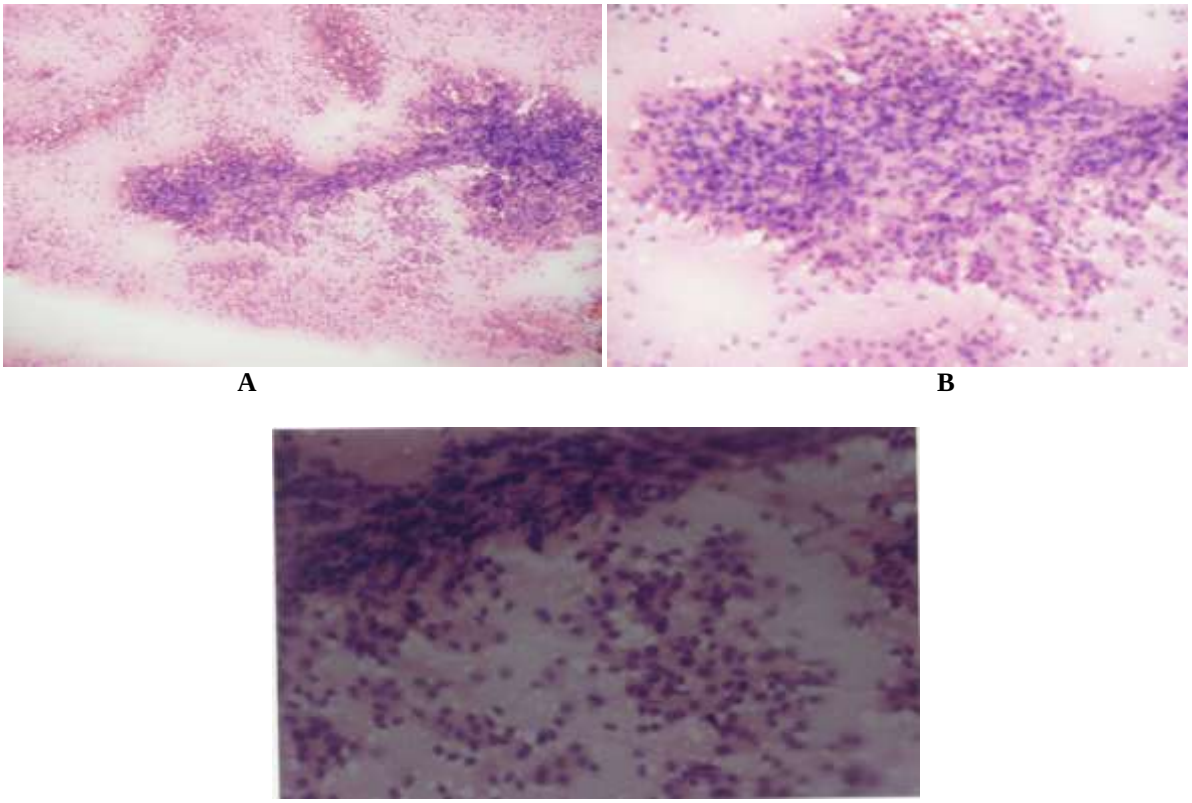
Figure 3: FNAC of Liver: Regenerating nodule.

Cytosmear shows Hepatocyte clusters showing several binucleate hepatocytes against hemorrhagic and necrotic background.(H & E, x400).

Of the 30 (75%) cases of malignant tumors, 2 cases (3.8%) were hepatoblastoma, 28 (53.8%) caseswere hepatocellular carcinomas and 10 (19.2%)cases were secondaries which are from colorectal adenocarcinomas. Ratio between primary and secondary is 3:1. Hepatocellular carcinoma was more common than secondaries in our study and males were affected more frequently.

Cytosmear from hepatoblastoma showed small round cells with variable amount of cytoplasm and fragments of mesenchyme.

HEPATOBLASTOMA



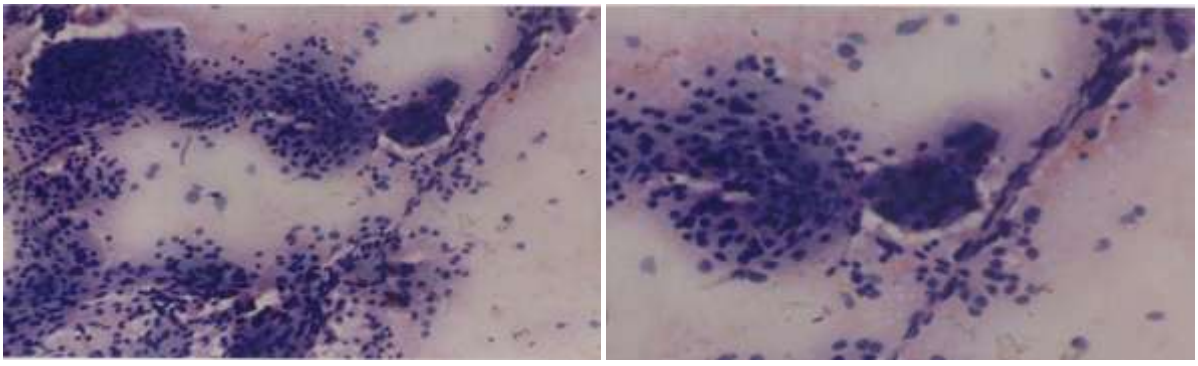
A **B**

C

Figure 4: FNAC of Liver:Hepatoblastoma – A – Hepatoblastoma, B&C-Cytosmear shows small round cells with variable amount of cytoplasm and Fragments of mesenchyme. (A x40, B x100, C x400).

Hepatocellular carcinoma was seen in 28 (53.8%) cases. This is the most common tumor which we encountered in our study. It is divided into three categories based on differentiation-well differentiated 7(13.45%), moderately differentiated 9(17.29%) and poorly differentiated 12(23.05%). Well differentiated tumors showed Polygonal neoplastic cells arranged in trabeculae, acini and sheets with abundant eosinophilic granular cytoplasm, high N:C ratio. Endothelial cells traverse cell groupings. Bile plugs seen in between cells. Bile duct epithelium was present in few cases. Moderately differentiated cells showed smaller sheets, many single lying cells and occasional endothelial cells. Poorly differentiated hepatocellular carcinomas showed frequent single cells with all features of malignancy. No endothelial cells were seen in this type.

HEPATOCELLULAR CARCINOMA (HCC)
WELL DIFFERENTIATED HCC



A **B**

Figure 5: FNAC of Liver:Well differentiated HCC. A&B-Cytosmear shows neoplastic cells arranged in trabeculae, acini and sheets. Cells are polygonal in shape with central nuclei and abundcanteosionophilic granular cytoplasm with high N/C ratio There are few atypical striped malignant nuclei with inclusions. Endothelial cells traversing cell groupings. Bile plugs are seen in between cells.(H&E A x100, B x400).

MODERATELY DIFFERENTIATED HCC

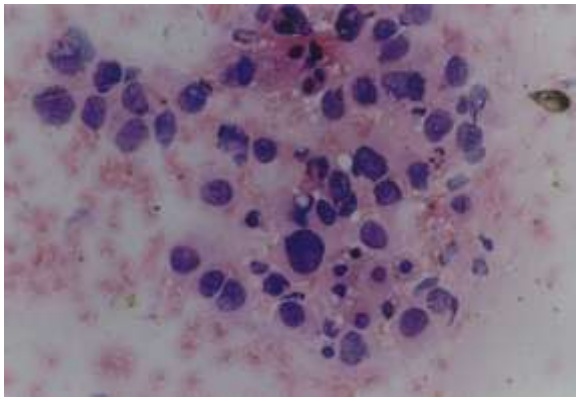
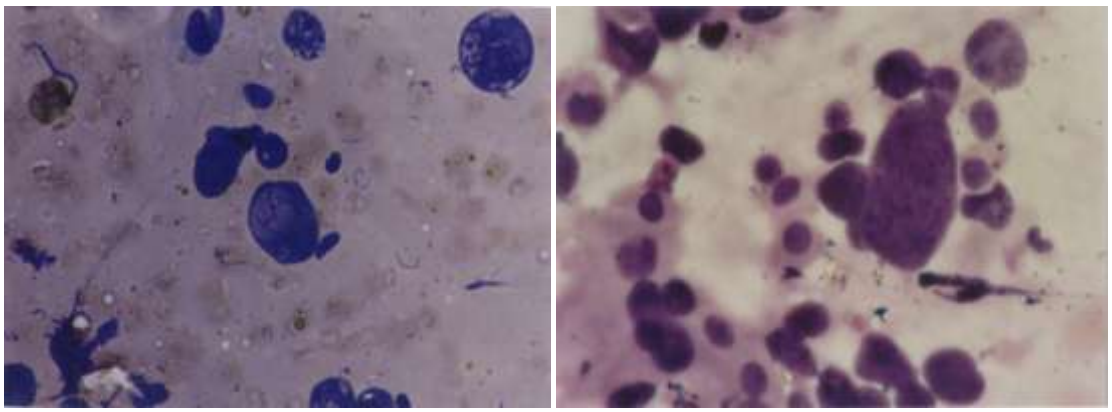


Figure 6: FNAC of Liver: Moderately differentiated HCC. Cytosmear shows smaller sheets of pleomorphic cells. Single lying cells with occasional endothelial cells against hemorrhagic background.(H&E, x400)

POORLY DIFFERENTIATED HCC

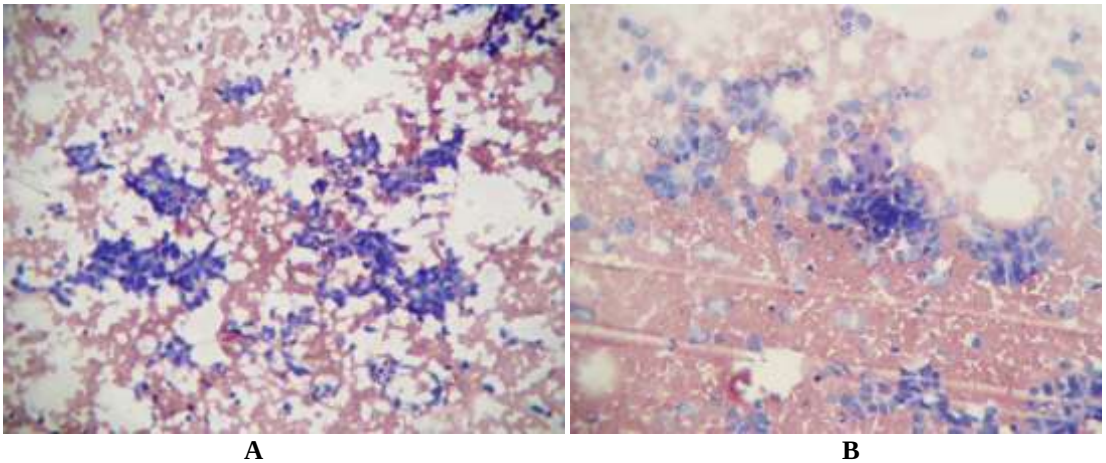


A **B**

Figure 7: FNAC of Liver: Poorly differentiated HCC. A&B – Cytosmear shows mainly single lying cells with pleomorphism, hyperchromatism. Endothelial cells are not seen.(H&E, x400)

10 cases (19.2%) were secondaries which are from colorectal adenocarcinomas.Cytosmear shows malignant epithelial cells in micro glandular groups, cells showing vacuolated or granular cytoplasm with high N:C ratio. Background shows necrotic debris, hemorrhage and few benign hepatocytes. Normal hepatocytes are seen which is suggestive of metastasis within the Liver

SECONDARIES in LIVER



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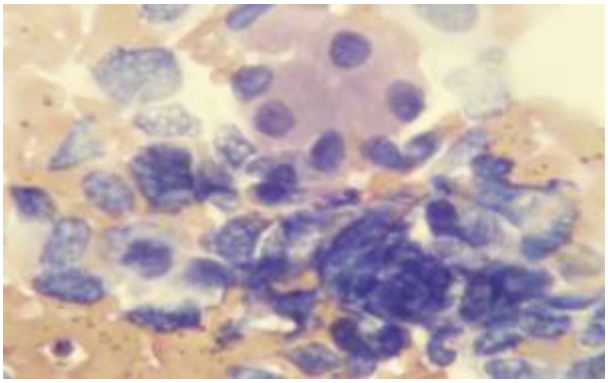

C

Figure 8: FNAC of Liver: Secondaries of the Liver. A, B,C – Cytosmear shows malignant epithelial cells in micro glandular groups against background of necrotic debris. Features suggestive of Adenocarcinoma. C – Normal hepatocytes are seen suggestive of metastasis within the Liver.(A x40, B x100,C x400).

DISCUSSION

Present study on Space Occupying Lesions(SOL)of liver is done on samples collected from a tertiary care center. We received 52 cases of liver SOL out of 3180 cases of FNACs. Percentage of liver SOL is 1.64 %. Commonest lesion is hepatocellular carcinoma-53.8 %. 21Gauge fine needle was used for aspirations of hepatic SOL. Our results were good with 21G fine needle. 86.54% of cases were adequate for opinion and only 13.46% were inadequate for opinion which shows good sensitivity (86.5%), specificity (94.3%) and diagnostic accuracy (100%). Li GP et al conducted study in which they compared the total positive rate (TPR) and safety for both groups i.e. 21G fine needle and 18G Tru-cut core needle. TPR was same in both groups. Liver puncture tract subcapsular hemorrhage and arteriovenous shunt were reported with 18G Tru-cut core needle. Even pain was higher with 18G Tru-cut core needle.[5] We dint use 18G Tru-cut core needle for any of our cases.

Most of our cases were diagnosed by FNAC alone. Histopathological confirmation was needed only in three cases of poorly differentiated HCC which were misdiagnosed as metastasis. 7 cases were inadequate for opinion. FNAC is gold standard for both neoplastic and non-neoplastic lesions. The only problem is diagnosing the poorly differentiated HCC as most of the metastatic adenocarcinomas also present with similar cytologic features. In a study done by Kacar et al in which they diagnosed their cases first by FNAC and later confirmed by histopathological analysis. 15 of 22 HCC cases were diagnosed by FNAC alone. The primary site was unknown in 28 of the metastatic tumors in their study.[6]

US-guided FNAC offers accuracy without major complications and minimal intervention at a lesser cost.[12] Imaging technique is useful in diagnosing abscess, but the problem arises when there is necrosis in the malignant tumor and the aspirated material is necrotic debris and misdiagnosed as abscess. In these situations FNAC plays an essential role.[13]The same thing is for regenerating nodule for which clinical diagnosis was hepatocellular carcinoma.Cytosmear from hepatoblastoma showed small round cells with variable amount of cytoplasm and fragments of mesenchyme. In a study done by Das C et al for FNAC of pediatric hepatic mass 12.9% of the pediatric hepatic tumors were hepatoblastoma. This is based on only pediatric FNAC lesions.[4] Our study includes cases of all ages and we got 2 (3.8%) cases of hepatoblastoma. In our study we made an attempt to classify hepatocellular carcinomas into well, moderate and poorly differentiated carcinomas based on the criteria laid down by Bottles K et al and Pitman MB et al. Table 3 shows the comparison of cytomorphological features of HCC of our study with Wee A et al study.[13]

Hepatocellular carcinomas which is the most common primary malignant hepatic tumorin our study was divided into three categories based on differentiation. This differentiation is based on primary and secondary criteria laid down by Bottles K et al[14] and Pitman MB et al.[15] In his study Bottles K et al used key diagnostic criteria and secondary criteria to differentiate hepatocellular carcinoma. Polygonal cells with centrally placed nuclei, malignant cells separated by sinusoidal capillaries and bile as key cytologic criteria for HCC whereasendothelial cells surrounding tumor cell clusters and intranuclear cytoplasmic inclusions were taken as secondary criteria. Well differentiated tumors showed Polygonal neoplastic cells arranged in trabeculae, acini and sheets with abundant eosinophilic granular cytoplasm, high N:C ratio. Endothelial cells traverse cell groupings. Though Bile is seen, none of our cases show bile duct epithelium. Moderately differentiated cells showed smaller sheets, many single lying cells and occasional endothelial cells. Poorly differentiated HCC showed frequent single cells with all features of malignancy. No endothelial cells and no bile duct epithelium seen in this type. So for the majority of patients of HCC there is no problem for diagnosis.

In a Wee A et al study for differentiation of HCC with diagnostic pitfalls, hypercellular aspirates composed of cohesive clusters of atypical hepatocytes with arborescent, tongue-like projections of broad trabeculae, with or without peripheral endothelial rimming are

pathognomonic of classic HCC. As the tumor grade increases, there is corresponding increase in cellular dissociation, with less evidence of transgressing and peripheral endothelium and fewer trabeculae.[13]

Hypercellularity, broad trabeculae, endothelial transgressing and bile duct epithelium is more common in well differentiated HCC. Dissociation, anisocytosis, increased N: C ratio, eccentric nucleus, anisokaryosis, bare nuclei with prominent nucleoli is seen more in higher grades of tumors i.e. moderate and poorly differentiated HCC.

Comparasion of Cytological features

Table 3: Comparison of cytological features of Present study with Wee et al study

Cytological features	Wee et al.,			Present Study		
	W %	M %	P %	W %	M %	P %
Hypercellularity	100	82	80	100	84	78
Dissociation	81	90	100	75	88	100
Broad trabeculae (>3 cell thick)	81	74	20	88	67	28
Narrow cords (< 2 cell thick)	100	87	60	100	66	27
Peripheral endothelium	48	36	10	78	48	0
Transgressing endothelium	100	87	30	100	72	38
Pseudo-acini	43	33	10	54	38	0
Intracytoplasmic inclusion	67	36	40	32	28	18
Bile	19	26	0	24	18	0
Bile duct epithelium	43	38	10	0	0	0
Anisocytosis	43	95	100	44	96	100
Increased N:C ratio	100	100	100	100	100	100
Well-defined cytoplasmic border	52	28	0	64	38	0
Eccentric nuclei	62	95	100	74	96	100
Anisokaryosis	62	100	100	58	100	100
Intranuclear inclusion	48	33	20	38	24	16
Multiple nuclei (>3)	67	72	80	42	62	84
Naked nuclei	62	87	100	54	88	100
Multiple nucleoli (>2)	38	67	80	34	68	82
Large nucleoli	62	77	90	24	68	100

W- Well differentiated HCC, M – Moderately differentiated HCC, P – Poorly differentiated HCC, HCC – hepatocellular carcinoma

Bile and bile duct epithelium is seen mostly in well differentiated and moderately differentiated HCC but rare in poorly differentiated HCC.[13] In our study, the cytosmear shows bile but bile duct epithelium was absent. Intracytoplasmic inclusions were present in 18-32% of HCCs, their presence strongly suggest hepatocellular carcinoma. But these types of inclusions are also seen in other tumors like ovarian, lung, adrenal gland and asbestosis of lung.[16] Atypical naked nuclei were seen more commonly in poorly differentiated HCCs.[17]

In a study conducted by Balani S et al, they evaluate the cytomorphological features of hepatocellular carcinoma only without focusing on other tumors. In our study we evaluate both malignant and non-malignant lesions.[7] In a similar study conducted by Nggada HA et al for FNAC diagnosis of liver diseases, their study revealed hepatocellular carcinoma to be the most common lesion similar to our study. But they dint focused on grading of HCC and also nonmalignant lesions.Our study included both malignant and nonmalignant as well as grading of HCC.[8]In a study conducted by Swamy MC et al,for value of US guided FNAC in the investigative sequence of hepatic lesions with emphasis on HCC.They classified lesions into benign and malignant. They found hepatocellular carcinomas to be most common lesion followed by metastatic adenocarcinomas.[9] In a similar study done by Nazie RT et al, they also got same results i.e. HCC was the commonest malignancy.[10] However in both these studies grading was done. In a study done by Mrzljak A et al for the role of FNAC in the management of hepatocellular carcinoma, in this study they classified only hepatocellular carcinoma without focusing on non-neoplastic conditions and secondaries. They classified their patients into cirrhotic and non-cirrhotic. One observation in this study was, FNAC can be done in patients with advanced liver diseases with ascites and also in patients with bleeding disorders.[11] In our study we classified all types of space occupying lesions including non-neoplastic lesions. In our study also they were patients with advanced disease with prolonged

clotting and bleeding time. We did FNAC in these patients without any complications.

Difficulty in diagnosis arises in two situations. Firstly to differentiate between well differentiated HCC with Adenoma and secondly poorly differentiated HCC with metastasis.[18] In a study done by Cohen et al and Bottles K et al, they did stepwise logistic regression analysis to differentiate HCC and reactive liver cells.[19] and between HCC and metastatic tumor cells.[14] In study done by Soyuer I et al and Wee A et al, they put forward certain criteria to distinguish Well differentiated HCC with reactive liver cells. These criteria are: Architectural features, hyper cellularity, arborescent cohesive clusters, broad trabeculae, endothelium, small monotonous hepatocytes, increased N: C ratio, cytoplasmic inclusions, naked nuclei and tumor giant cells. Well defined cytoplasmic borders, abundant cytoplasm, eccentric nuclei, thick nuclear membrane, irregular nuclear contours, increased and irregular chromatin were not detectable always in well differentiated HCC. Some of these features are seen in dysplastic hepatocytes also.[18, 20]

The secondaries which we encountered were deposits of adenocarcinoma. Colon and rectum were the primary site. Cytosmear from secondaries showed malignant epithelial cells in micro glandular groups and cytoplasm contain abundant mucinwith background of necrotic debris.

In Bottles K et al study they used three criteria to differentiate HCC from metastatic tumors. These were 1. Polygonal cells with centrally placed nuclei 2.Malignant cells separated by sinusoidal capillaries and 3. Bile. Along with this there are two secondary criteria to diagnose HCC vizendothelial cells surrounding tumor cell clusters and intranuclear inclusions.[14]

In our study the cytomorphological features which are seen in metastatic carcinomas are malignant cells arranged in micro glandular groups, necrosis and presence of normal hepatocytes. Immunohistochemistry is of value if there is difficulty in differentiating poorly differentiated HCC with poorly differentiated secondaries.

Reddy CV et al in 2015 did a study and found that secondaries were the most common SOL of liver. One feature which is seen in this study was they got two patients who developed needle tract metastasis. None of our patients reported tract metastasis in our study.[1] In a study done by talukdar et al, secondaries were more common than primary.[2]

The smears which were inadequate for opinion consists mostly of blood, blood elements, necrotic debris and or consists of normal hepatocytes even after repeated aspirations. In the study done by Barbhuiya M et al in 2014 for FNAC of liver, 9% of cases were inadequate for opinion. They got necrotic debris and reactive hepatocytes in these types of smears.[3]

Diagnosis of Space occupying lesions of liver is difficult with any single diagnostic method as is proved in our study. Its a multidisciplinary team approach which comprises of hepatologist, radiologist and cytopathologist.

The sensitivity, specificity and diagnostic accuracy of US guided FNAC of liver in our study (86.5%, 94.3%, 100%) is similar to other studies. Reddy CV et al [1] (93%,90.9%,92.8%). Das C et al [4] (94%,93%,93.10%).Kuo FY et al [21] (85.1%, 98.7%, 86.1%). When samples with inadequate for opinion are excluded our study shows diagnostic accuracy of 100%.

CONCLUSION

Space occupying lesions of liver requires accurate diagnosis for proper management and prognosis. Ultrasound guided FNAC is a useful, easy, uncomplicated and cost effective procedure in the diagnosis of Space occupying lesions of Liver. Most studies favor FNAC with 21G fine needle over 18G tru-cut core needle. Fine needle aspirations can be taken from both left and right lobes and from superficial or deep lesions.FNAC is useful procedure in patients with advanced liver disease and with coagulation disorders. Since there is no hemorrhage after the procedure, it can be done in patients with bleeding disorders. One more advantage of using fine needles and cytological techniques is that smears can be stained and checked immediately and the biopsy repeated from different sites in the liver until a satisfactory sample is obtained without any risk to patient. In this way diagnostic accuracy can be increased. The sensitivity, specificity and diagnostic accuracy of present study is 86.5%,94.3%, and 100% respectively.

SOURCE OF SUPPORT: Nil

CONFLICT OF INTEREST: None declared.

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