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Cytological Analysis of Deep-seated Abdomino- Pelvic masses in an image-Guided Fine Needle Aspiration Cytology and Correlation with Clinico-Cytological Parameters

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ABSTRACT

Background: The image-guided fine needle aspiration cytology in deep-seated intra-abdominal and pelvic masses has become the optimal procedure to establish tissue diagnosis for their appropriate management. Aim: The aim of the present study was to evaluate the adequacy rate of image-guided FNA and to analyze the cytological spectrum of abdominal-pelvic masses with emphasis on hepatic lesions and correlation with Clinico-cytological parameters

Material & Methods: This was a prospective study conducted in the Department of Pathology from July 2020 to July 2023 comprising 280 patients with intra-abdominal and pelvic masses who underwent image-guided FNA. Results: The cytological analysis revealed 89.3% smears as adequate for evaluation. The adequate aspirations were categorized as neoplastic(94.4%) and non-neoplastic (5.6%) which were further categorized as hepatocellular carcinoma (18%), metastases to the liver(60%), adenocarcinoma of the gallbladder (6.4%), Malignant lymphoma in intraabdominal lymph nodes(2.8%), pancreatic adenocarcinoma (2%), ovarian adenocarcinoma(1.6%), retroperitoneal malignant mesenchymal tumours (1.2%), renal cell carcinoma(1.2%), adenocarcinoma colon(1.2%) xanthogranulomatous cholecystitis (1.6%), granulomatous liver abscess(0.8%), pyogenic liver abscess (0.8%) and reactive hepatocytes (2.4%). An association between the size of the lesion and adequacy of FNAC aspirations is found with the highest number of inadequate aspirations (92%) in the size of the lesion (0.1<1.0cm) followed by 71.4% in the group where size the of the lesion was 1<2cm. We observed a statistically positive correlation of cytological diagnosis with gender, radiological appearance, HBsAg positivity as well as history of alcohol intake ($p < 0.000$). Conclusion: The image-guided FNAC is an efficient, safe, minimally invasive, low-cost outpatient procedure for the diagnosis of deep-seated abdominal and pelvic masses.

Keywords: Ultrasound/CT guided fine needle aspiration cytology, intra abdominal masses, pelvic masses.

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Introduction.

The image-guided fine needle aspiration has gained tremendous acceptance as a diagnostic procedure for deep-seated abdominal and pelvic space-occupying lesions. The clinical data, radiological parameters and serum tumor markers can help narrow the differential diagnosis, but tissue diagnosis remains the gold standard. It helps clinicians choose appropriate patient management, ranging from supportive care for advanced metastatic lesions to surgical resection for primary carcinoma and radiotherapy/chemotherapy for lymphoproliferative disorders. The optimal results are obtained with a dedicated and experienced cytopathologist and radiologist team and close clinic-cytological correlation.

The present study aims to evaluate the adequacy rate of image-guided FNA and analyze the cytological spectrum in abdominal and pelvic masses with emphasis on hepatobiliary masses along with clinical, biochemical and radiological correlation of image-guided FNAC.

Materials and Method:

This was a prospective study from July 2020 to July 2023 comprising 280 patients with abdominal and pelvic space-occupying masses who underwent image-guided FNA. Initially, clinical histories of the patients were taken and then thorough clinical examination and relevant laboratory investigations including coagulation profile were performed in all the patients. In addition radiological parameters in relation to size as well as number of lesions and biochemical details (serum bilirubin, serum aspartate, serum alanine and serum alkaline phosphatase were also recorded in all patients. The patient's consent was taken prior to the procedure. The FNAC was performed under ultrasound guidance in relatively superficial masses and computed tomography guided in deep-seated masses using a 21-23 gauge lumbar puncture needle, fitted to a 20 ml disposable syringe attached to a metallic syringe holder. In our institution, we have a team of radiologists and cytopathologists who in synergy carry out the procedure as a routine two passes were made to get adequate aspirate. Direct air dried smears were prepared for May Grunwald Giemsa Biolab Diagnostics Mumbai India and a few smears were immediately fixed in 95% alcohol for Haematoxylin and Eosin stain.

The Hepatitis B surface antigen, anti-hepatitis C virus antibody and alpha-fetoprotein were determined by an enzyme-linked immune absorbent assay in patients with hepatobiliary lesions. The stained FNA smears were examined by two cytopathologists independently for cytomorphological findings, diagnosis and differential diagnosis where needed. The SPSS version 17 software IBM was used for statistical analysis, one-way analysis and ANOVA were used to find an association between cytological diagnosis and biochemical parameters (serum bilirubin, AST, ALT, ALP and AFP). The association between the cytological diagnosis and clinical radiological parameters was assessed by means of a chi-square test. A probability value of 0.05 or less was considered statistically significant.

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Results

During the present study 280 patients underwent ultrasound/CT-guided FNA of abdominal and pelvic masses. The cytological smears were categorized into two groups: Group 1-Adequate aspirations (250, 89.3%). Group 2- Inadequate aspirations (30, 10.7%). The adequacy criteria as specified by Lester et al[1] was followed. The satisfactory smear had six or more cell clusters. Further cell cluster was defined as having five or more cells.

The adequate aspirations (250,89.3%) were categorized as Neoplastic (236,94.4%) and Non Neoplastic(14,5.6%).An association between the size of the lesion and adequacy of FNAC aspirations is found with the highest number of inadequate aspirations (92%) in the size of the lesion (0.1<1.0cm) followed by 71.4% in the group where size the of the lesion was 1<2cm. A Chi-square statistic shows that the size of the lesion has a significant level of association with the adequacy of the smear (p-value <0.001) as shown in Table no 1.

Table no 1

Correlation of size lesion with adequacy rate

Sr. No.	Size of lesion in cm	Adequate aspirations %	Inadequate aspirations%	Total
1	0<1 cm % with in-group	2 (8.0%)	23 (92.0%)	25 (100%)
2	1<2 cm % with in-group	2 (28.6%)	5 (71.4%)	7 (100%)
3	2<3cm % within group	85 (98.8%)	1 (1.2%)	86 (100%)
4	3<4 cm % within group	87 (98.9%)	1 (1.1%)	88 (100%)
5	4<5cm % within group	40 (100%)	0	40 (100%)
6	5<6 cm % within group	10 (100%)	0	12 (100%)

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7	>7 cm % within group	24 (100%)	0	24 (100%)
Total **X ^{2} =225.166; df=6 ,p= <o.oo1**		**250; 89.3%**	**30, 10.7%**	**280,100%**

P<0.0001; Highly significant

The distribution of various Neoplastic and Non Neoplastic lesions were hepatocellular carcinoma (45,18%), metastases to the liver (150,60%), adenocarcinoma of the gallbladder (16,6.4), Malignant lymphoma of intraabdominal lymph nodes(7,2.8%), pancreatic adenocarcinoma (5,2%), ovarian adenocarcinoma(4,1.6%), retroperitoneal malignant mesenchymal tumours (3,1.2%), renal cell carcinoma(3,1.2%), adenocarcinoma colon(3,1.2%) xanthogranulomatous cholecystitis (4,1.6%), granulomatous liver abscess(2,0.8%), pyogenic liver abscess (2,0.8%), reactive hepatocytes (6,2.4%) and as summarized in table no2**.** The male predominance was seen in all malignancies except in adenocarcinoma gall bladder, which was more frequent in females in our study. We observed neoplastic lesions occurred more frequently in patients older than 60 years in contrast non-neoplastic lesions involved patients less than 60 years and. In the HCC category, 40 patients (88.88%) had a solitary space-occupying lesion in contrast the majority of patients (96.6%) diagnosed with metastases to the liver presented with multiple space-occupying lesions. Cirrhosis of the liver was radiologically documented in 53.33% of HCC patients. However, none of the patients with metastases, adenocarcinoma gall bladder and non-neoplastic lesions had cirrhosis. The size of the lesion varied from 2 to 12 in patients with HCC. We observed a statistically positive correlation of cytological diagnosis with gender, radiological appearance (single /multiple lesion and cirrhosis), HBsAg positivity as well as history of alcohol intake ($p < 0.001$).

Table no 2

Correlation of cytological diagnosis with gender, age, radiological appearance, cirrhosis, history of alcohol intake and HBsAg positivity and HCV positivity.

Sr. No.	FNAC diagnosis	Gender		Age		No: of lesions		Cirrhosis	Alcohol	HBsAg	HCV
		M	F	<60	>60	ML	SL				
1	Hepatocellular carcinoma (n- 45)	35	10	19	26	5	40	24	23	30	5
2	Metastases Liver (n-150)	109	41	27	123	145	5	0	15	8	1
3	Adenocarinoma Gall bladder (n-16)	4	12	5	11	1	15	0	0	0	0

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4	lymphoma lymph node (n-7)	2	5	2	5	6	1	0	0	3	1
5	Pancreatic adenocarcinoma (n-5)	4	1	2	3	1	4	3	3	2	0
6	Ovarian adenocarcinoma (n-4)	0	4	2	2	0	4	0	0	0	0
7	Retroperitoneal malignant mesenchymal tumor (n-3)	2	1	1	2	0	3	0	0	0	0
8	Renal cell carcinoma (n-3)	1	2	1	2	0	3	0	0	0	1
9	Adenocarcinoma colon(n-3)	2	1	0	3	1	2	0	0	1	0
10	Xanthogranulomatous cholecystitis (n-4)	1	3	3	1	0	4	0	0	0	0
11	Liver Tubercular abscess (n-2)	1	1	1	1	1	1	0	0	0	0
12	Liver pyogenic abscess (n-2)	1	1	1	1	1	1	0	0	0	0
13	Reactive hepatocyes(n-6)	5	1	5	1	1	5	0	0	0	0
	P VALUE	**<0.001****		**0.004***		**<0.001****		**<0.001****	**<0.001***	**<0.001****	**0.014***

***p<0.05; Significant; **p<0.001; Highly significant ML: multiple lesions,SL: single lesion, HBsAg: Hepatitis B surface antigen, anti-HCV: Anti hepatitis C antibody**

Hepatobiliary lesions

Hepatitis B surface antigen and anti-hepatitis C virus positivity were seen in 66.67% and 11.11% of cases of HCC. In the Hepatobiliary neoplastic group biochemical parameters (serum bilirubin, serum AST, serum ALT, serum ALP) were raised in all categories in contrast serum AFP was specifically raised in patients of hepatocellular carcinoma (p<0.001) as shown in table no 3 . However AFP showed no statistical correlation with the degree of differentiation of HCC (p=0.075). The cytological features which helped in distinguishing HCC from metastases and primary carcinoma gall bladder were a trabecular arrangement of tumour cells (82%), transgressing vessels through tumour cells (84%) fig1a, endothelial wrapping of tumour cells (30%) fig1band 1c, polygonal cells with central nuclei and abundant granular cytoplasm (95%), intacytoplasmic bile (35%) fig 1d, intracytoplasmic inclusions (15%) fig2a, high N/C ratio(88%), macronucleoli nucleoli(52%) fig2b, multiple nucleoli (55%), atypical naked nuclei (84%) and presence of hyaline globules (22%). The HCC was classified into well differentiated (6 cases),

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moderately differentiated (32 cases) and poorly differentiated to poorly differentiated (7 cases).

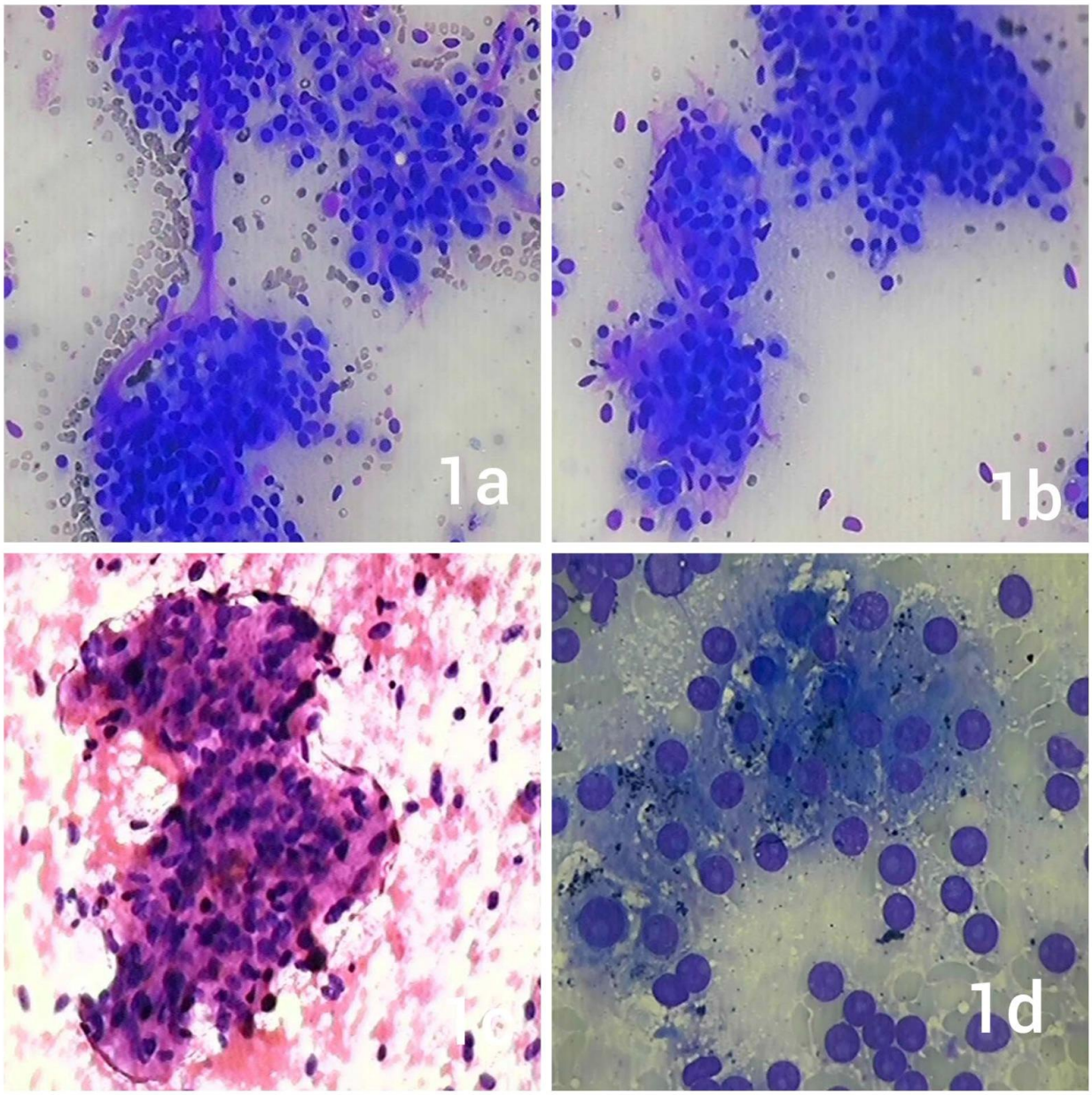

Fig 1a Giemsa stained smear shows transgressing vessel through cluster of hepatocellular carcinoma (x40)

Fig 1b Endothelial wrapping around a cluster of tumour cells of hepatocellular carcinoma (Giemsa X40)

Fig 1c Endothelial wrapping around a cluster of tumour cells of hepatocellular carcinoma (Hand E x40)

Fig 1d Intracytoplasmic bile pigment in case of hepatocellular carcinoma (Giemsa x40)

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Table 3

Correlation of cytological diagnosis in hepatobiliary lesions with serum bilirubin, Asparate, Alanine, Alkaline phosphatase and Alfa feto proteins

Sr. No.	FNAC DIAGNOSIS (subtype)	Bilirubin		AST		ALT		ALP		AFP	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1.	Hepatocellular carcinoma (n-45)	4.980	1.9992	143.111	126.313	142.755	123.785	225.00	137.007	726.111	201.764
a.	Well-differentiated (n-6)	6.217	2.0827	252.500	176.256	240.500	158.366	343.667	195.797	847.667	214.347
b.	Moderately differentiated (n-32)	5.125	1.9913	140.625	117.359	142.031	119.921	223.468	125.313	731.781	208.745
c.	Poorly differentiated (n-7)	3.257	0.3207	60.714	5.908	62.285	7.250	130.285	3.401	596.000	20.265
2.	Metastasis Liver(n-150)	3.395	1.1543	75.440	27.998	83.366	34.778	153.253	33.563	0.690	0.743
3.	Adenocarcinoma Gall bladder (n-16)	2.694	0.3235	44.625	5.239	48.875	6.601	153.250	15.997	0.312	0.182
4.	Xanthogranulomatous Gall bladder (n- 4)	0.375	0.1708	27.000	2.581	31.500	1.914	61.250	8.539	0.200	0.081
5.	Tuberculosis liver (n-2)	0.200	0.000	31.000	1.414	34.000	0.000	62.500	3.535	0.350	0.070
6.	Pyogenic liver abscess (n-2)	0.250	0.0707	34.000	0.000	33.00	1.414	67.000	1.414	0.200	0.000
7.	Reactive hepatocytes (n-6	0.400	0.000	32.33	1.366	33.333	1.032	66.000	1.788	0.416	0.256
	Total	26.893	8.1132	841.345	464.434	851.645	454.679	1485.673	526.354	2903.727	646.453
		P<0.001		P<0.001		P<0.001		P<0.001		P<0.001	

p<0.001; Highly significant

serum bilirubin, AST: Aspartate, ALT: Alanine,ALP: Alkaline phosphatase and AFP: Alfa to proteins

The metastatic tumours to the liver constituted 60% of all aspirates and metastatic adenocarcinoma was the commonest (80%) Fig 2c. The primary sites of adenocarcinoma were the gastrointestinal tract (85%), ovary (5%), prostate (5%), breast (2%) and unknown (3%). The smears of the adenocarcinoma group exhibited hypercellularity with tumour cells arranged as a gland, acini and multilayered cell clusters. The cells exhibited altered N/C ratio and anisonucleosis with centrally/eccentrically placed nuclei. Intracytoplasmic mucin (20%)andnecrosis (10 %.) were

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also observed in this group.

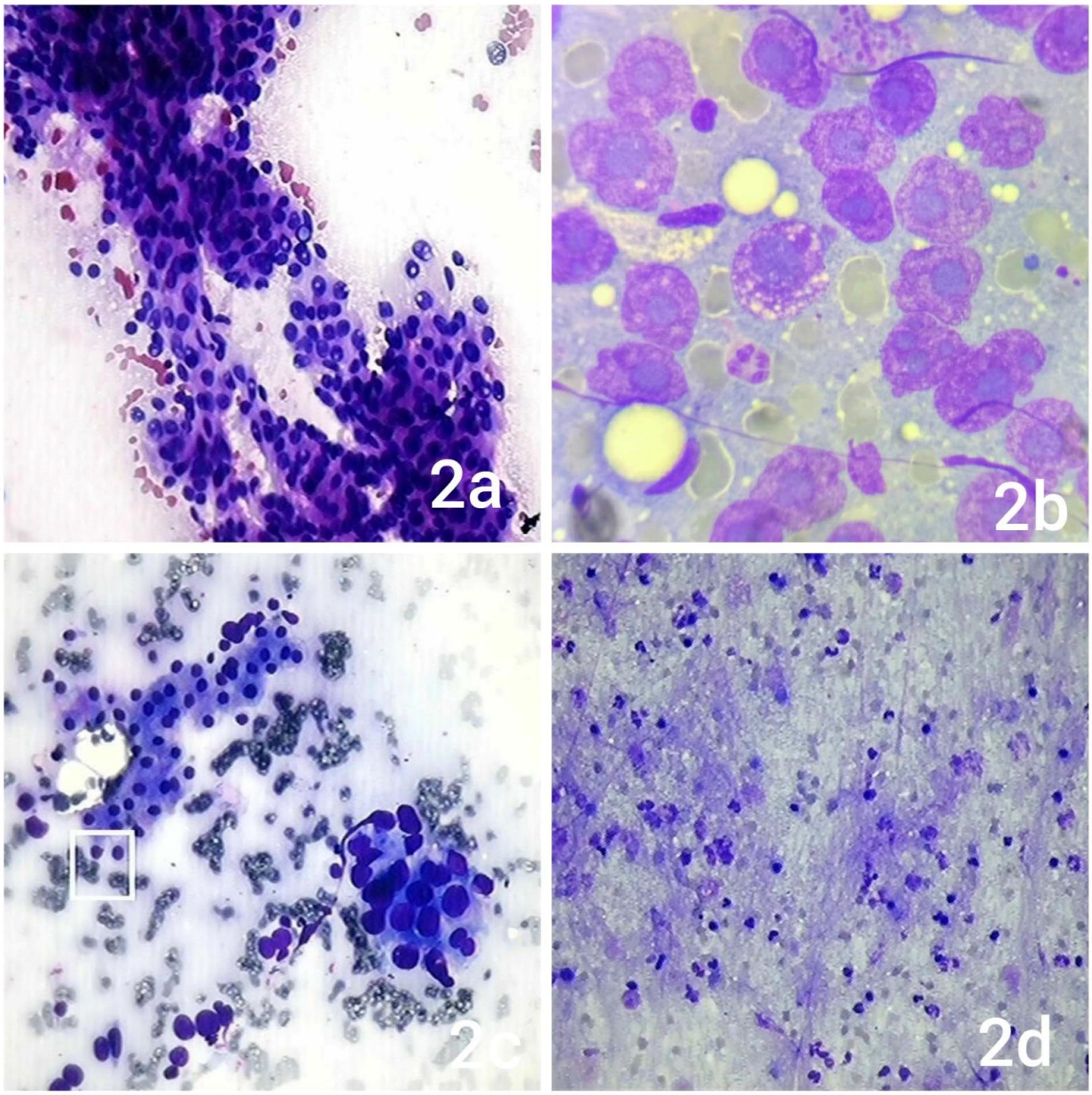

Fig 2a Smear shows intracytoplasmic inclusion in a cluster of hepatocellular carcinoma (Giemsa x40)

Fig 2b Giemsa stained smear shows macronucleoli in hepatocellular carcinoma (Giemsa x40). Fig 2c Giemsa stained smear shows a cluster of metastatic deposits in the liver (Giemsa x40).
Fig 2d of pyogenic liver abscess comprising polymorphonuclear neutrophils and cell debris (Giemsa x40)

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The smears from pyogenic liver abscess predominantly comprised polymorphonuclear neutrophils fig2d. The aspirates from granulomatous liver abscess showed epitheliod cell granulomas.

Discussion

The role of image-guided FNA in the diagnosis of abdominal and pelvic masses has evolved over the years. The main role of diagnostic cytology is to confirm malignancy by excluding the inflammatory lesions and further help in differentiating primary tumours from secondary. The cytological analysis in the present study revealed 89.3% as adequate for evaluation and 10.7% as inadequate for evaluation. Smith et al[2], Sidhalingreddy et al[3] and Bell et al [4 had] inadequacy rates of 4%, 7.3% and 13 % in their respective works. In the present study as shown in Table no.1 and described above it was observed that lesions with size 0.1cm-<1.0cm had the highest number of inadequate aspirations (92%) indicating relative difficulty in obtaining material from smaller. This is in concordance with results obtained by Barbhulya M et al[5] in their work on 400 aspirates of the liver and gall bladder.

Hepatobiliary lesions: In our study, liver aspirates were highest in number and comprised of 82% of all intra-abdominal FNACs. Similarly, in studies by Smith et al[2] and Sidhalingreddy[3], the liver was the commonest abdominal site. In the present analysis, the age group affected was between 45 to 82 years with the mean age of presentation being 61 years. However, Ahuja et al[6] and Talukder et al[7] reported slightly lower mean age of the presentation of HCC. A higher incidence was seen in males, with male to female ratio of 2.3:1.Alpana Banerjee et al[8] and Nggada et al[9] analyzed hepatic aspirates over the period of two years and showed almost similar M: F ratio of 2.3:1 and 2.5:1 respectively. We observed metastases to the liver in 60% cases followed by HCC in 18%, primary adenocarcinoma gall bladder in 6.4, xanthogranulomatous cholecystitis in1.6%, granulomatous liver abscess (0.8%), pyogenic liver abscess (0.8%) and benign reactive hepatocytes in 2.4% cases. This is in concordance with a study conducted by Jitendra et al[10] who reported 66.67 as metastases, 27.33% as HCC, 3.3% as benign and 2% as inflammatory. Tao et al[11] in their work on 1383 cases of hepatic aspirates reported 1037 (75%) as metastatic deposits. The current analysis showed that various biochemical parameters (bilirubin, AST, ALT, ALP) were raised in all hepatobiliary lesions. Similar results were obtained by Banerjee A et al [8] and Thapa BR[12]. These biochemical parameters act as non-specific indicators and might suggest an underlying chronic injury to the liver.

However present analysis showed increased alpha-fetoprotein in all cases of HCC in contrast none of the cases of metastases and primary adenocarcinoma of gall bladder exhibited elevation of AFP. Similar results were obtained by Radhika NS et al[13] and Banerjee A et al [8]where AFP was elevated in 81.8% and 75% of cases respectively. In the present work, no association was found between degrees of differentiation of HCC and elevation of AFP levels (p=0.075) which is in concordance with a study conducted by Ahuja et al [6] (p=0.14). HBsAg positivity in cases of HCC was observed in 66.66% of aspirates in our study. Studies conducted by Radhika NS et al [13] and Joshi N et al [14] reported 50-70% incidence of HBsAg positivity in HCC. The Hepatitis C virus is another major etiologic factor for HCC in Southeast Asian countries. In the current analysis, HCV antibody positivity was seen in 11.11% of patients with HCC, however, a slightly higher

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prevalence was reported by Ahuja et al [6] in their work on HCC. Cirrhosis was radiologically observed in 53.33% of patients with HCC. Similar results were obtained by Ahuja et [6] who reported an incidence of cirrhosis in 40% of cases of HCC.During the present analysis of cases of HCC, metastatic deposits in the liver and primary adenocarcinoma gall bladder presented as a single lesion in 88.88%, 3.33% and 93.75% respectively. Chhieng DC [15], and GSbolli et al[16] reported HCC as a solitary lesion in 82.5% and 80.48% of cases respectively.

The cytological features which helped in differentiating HCC from other malignancies have already been discussed, the presence of a predominant trabecular pattern with transgressing vessels was observed in 84% of cases of HCC. This is in concordance with studies conducted by Pitman et al[17], Cohen et al[17], Radhika NS et al[13] and Balani S et al [19]who reported trabecular patterns in HCC in 90%, 73%, 63.3%and 65% cases respectively. Endothelial wrapping around tumour cells was seen in 30% of cases in our study. Kung et al[20] and Tao LC et al [21] in their respective studies on hepatic aspirates concluded that the detection of endothelial wrapping around tumor cells is pathognomonic of HCC. Intracytoplasmic bile was observed in 35% of our cases. The presence of intracytoplasmic bile was seen in 17 to 68 % of the previously conducted studies from India[13, 23]. We observed a high N/C ratio in HCC was seen in 88 % of cases. Studies conducted by Mallikarjuna et al [24] and Cohen et al[18] showed that a high N/C ratio was the single most important feature favoring malignant hepatocytes. Binucleation and multinucleation were seen in 63% of cases of HCC. Ahuja et al[6] showed the presence of binucleation and multinucleation in 64% of cases. Macronucleoli nucleoli and multiple nucleoli were observed in 52 and 55 % of our HCC cases. This is in concordance with the results of Cohen et al [18] who reported prominent nucleoli and multiple nucleoli in 60 % and 54% cases. Atypical naked nuclei were observed in 84% of our cases of HCC. Pedio et al[25] and Cohen et al[18] revealed the presence of atypical nuclei in 93% and 73% of cases of HCC respectively. Eosinophilic intracytoplasmic hyaline globules were observed in a tiny proportion (22%) of our cases of HCC. This is in concordance with the findings of Mallikarjuna et al[24] who reported hyaline globules in 25% of cases of HCC. The adenocarcinoma was the most common tumour metastasizing to the liver in our analysis. This is in concordance with studies conducted by Pinto et al[26] and Das et al[27]. The salient features observed in metastatic adenocarcinomatous deposits in the liver were the glandular and acinar pattern of tumour cells exhibiting altered N/C ratio with the presence of intracellular mucin. Similar features of metastatic adenocarcinomatous deposits were highlighted by Greence et al[28] in their work on hepatic aspirates.

Mehdi et al[29] and Sidhalingreddy et al[3] studied 42 and 48 cases of ovarian tumors, of which 40% and 27% were malignant respectively. In our study, we had four cases of ovarian masses, all of which were malignant. We reported seven intra-abdominal lymph node aspirates as Malignant lymphoma. However, M Sheikhet al[30] in their work on 120 intra-abdominal and intra-thoracic masses observed a slightly higher percentage (10%) of lymphoma.

We had four cases of renal cell carcinoma in our study. Gangopadhyay et al[31] reported nine malignant renal cases, five of which were renal cell carcinoma, the rest being Wilms tumour.

Conclusion: Image-guided FNAC plays a pivotal role in the diagnosis of abdominal pelvic masses. It is a safe, minimally invasive, outpatient procedure, cost-effective and rapid method. Adequate

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sampling, high-quality smear preparation and experienced cytopathologists are crucial to the success and safety of the practice of FNA.

Conflicts of Interest: Nil

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