

**POST MORTEM LIVER F N A C
(FINE NEEDLE ASPIRATION CYTOLOGY)**

**THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF MEDICINE IN PATHOLOGY**

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I N T R O D U C T I O N

Fine Needle Aspiration Cytology [FNAC] is a simple inexpensive method for obtaining a tissue diagnosis of a palpable tumor(3)

It is a widely accepted routine method of preoperative diagnosis in most of the centers.

The FNAC procedure is very simple procedure, even it can be performed in out door without anaesthesia with multiple repeatation if required. The sensitivity of the procedure as compared to cutting biopsy and histopathology is 97 % - 99 %, usually it has no complications(21).

There are various techniques for FNAC as follows (4)....

- 1>Plane radiography or fluoroscopy.
- 2>Fluoroscopy plus contrast radiography.
- 3>U S G
- 4>Radionucleide scanning.
- 5>C T scan.

The liver is one of the most common organ involved by metastatic carcinoma as well as primary hepatocellular carcinoma. Detection of these neoplasms is important because of the obvious therapeutic and prognostic importance implications. Since many chemotherapeutic protocols require an accurate staging of disease, the liver is now frequently investigated for metastatic diseases by recently introduced radiologic techniques. These techniques although highly sensitive, are non specific. Therefore tissue confirmation of a suspected liver neoplasm is usually sought. (1)

Biopsy using the Meghini needle is a commonly used method for diagnosis of a diffused liver disorder, while useful, the method has some serious drawbacks. Complications may be observed and there also some contraindications such as liver tumour (due to increased Prothrombin time) and cardiac failure. In such cases FNAC is the method of choice. However, the reports of cytologic investigations are scarce and no attempt was made to correlate the cytologic and histologic findings in a diffuse liver disorders like cirrhosis, fatty change, and chronic diffuse hepatitis. (20)

This study represents an attempt to make such a correlation and ascertain the use of cytologic examination in diffuse liver disorders and malignancies (20).

There are few complications of liver FNAC such as ... Bile peritonitis (13,19) and implantation of tumour cells although very rare specially by large gauge needles (13).

A I M S A N D O B J E C T S

The purpose of the present study is

- 1> To find the cytomorphological features in various liver disorders
- 2> To assess the usefulness of the FNAC of liver in the diagnosis of various liver disorders by correlating the FNAC findings with histopathological findings.

REVIEW OF LITERATURE

FNAC is an inexpensive, atraumatic technique for the diagnosis of disease sites[3].

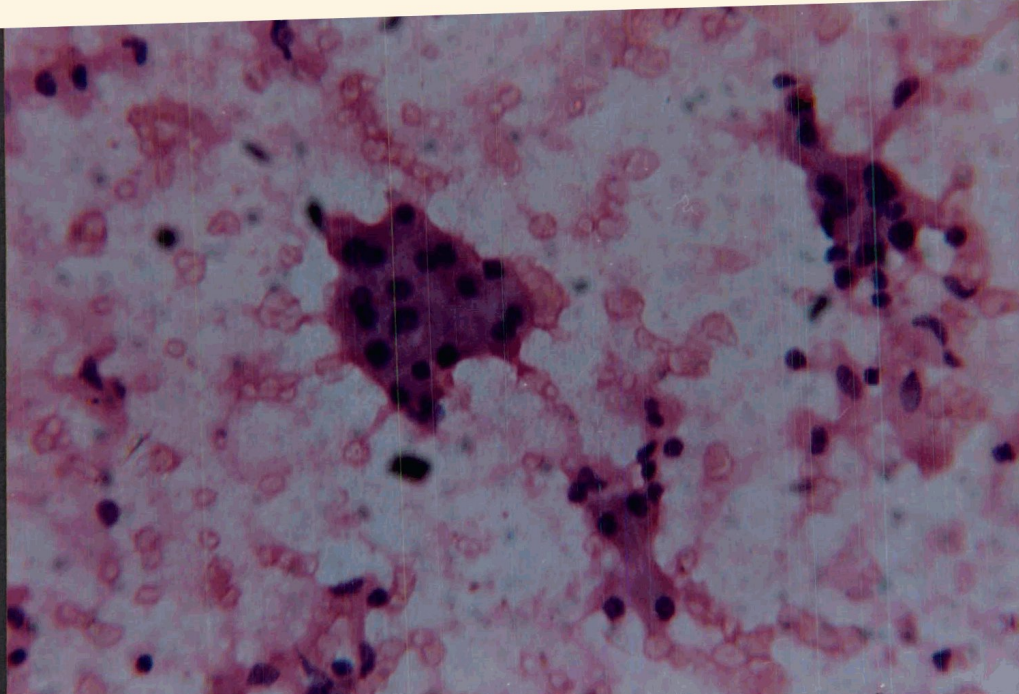
Kun in 1847 may have been the first person to report the use of aspiration biopsy [22]. In 1863 Fritchard used a grooved needle for the Breast aspiration and provided an excellent description of the cytology of Fat necrosis [3].

Koss[26] in History Of Cytology relates a number of events bearings on the development of diagnostic cytology and needle aspiration biopsy. He pointed out that Ewing did not like open surgical biopsies because he felt that they spread cancer [21]. Martin [27] needing a definitive diagnosis, compromised on aspiration, who used to practice aspiration on Cadavers. Dr. Fred Steward interpreted the aspiration biopsy samples & later reported a very large series with excellent diagnostic accuracy [26,27].

FNAB was first extensively used in Scandinavia [1].

The first paper on aspiration cytology from Memorial Hospital for Cancer & Allied Diseases was published in 1930 by Martin & Ellis [28].

In 1966, Soderstrom reported the first large series of FNAC of the liver, with 500 cases. This was followed by the 1971 report of 2611 liver aspirates by Lundquist & by 1973, Stormby & Akerman has documented 1435 FNACs of the liver [8].



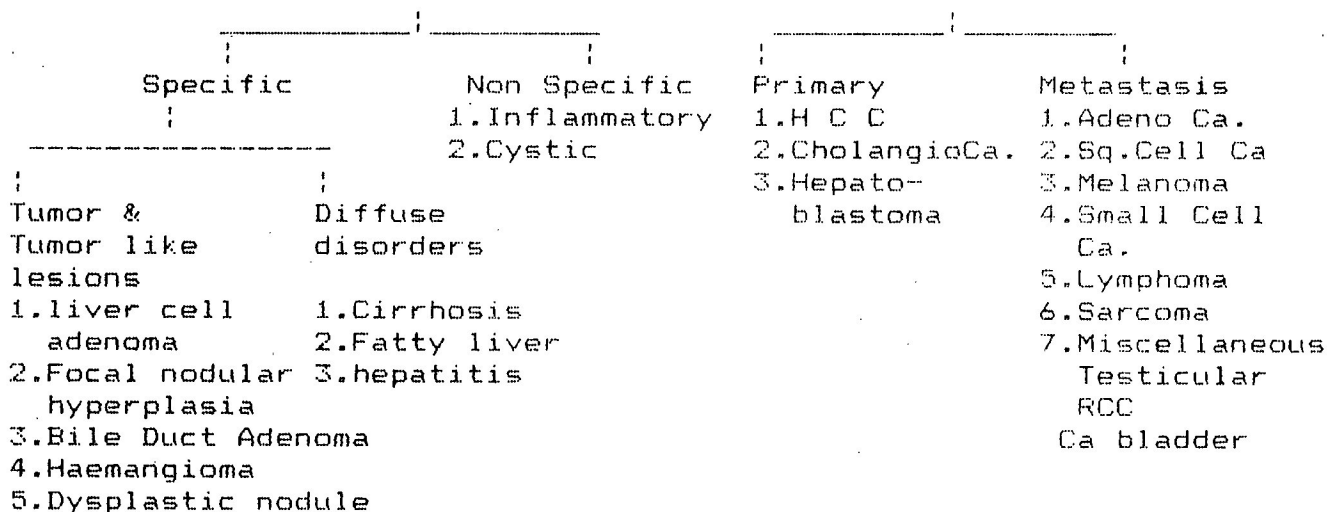
Smear showing normal Hepatocytes - H & E * 300

CLASSIFICATION OF ASPIRATES

1> Normal.

2> Benign.

3> Malignant



NORMAL LIVER CYTOLOGY

An asprate from normal liver tissue usually contain numerous hepatocytes and few bile duct epithelial cells, sinusoidal cells and kupffer cells are rarely seen [8].

Hepatocytes :

They are round to oval or polygonal with abundant granular eosinophilic or basophilic cytoplasm [8]. They occure singaly or in small monolayered sheets of 6-8 cells.

Nuclei are central round or vesicular and occupy about one quarter of the cell. The nuclear chromatin is granular and evenly distributed, they show one or two prominent round eosinophilic nucleoli [8].

Mitosis are almost never seen [8].

Bile duct epithelial cells :

Occur singly or in clusters of columnar cells that have rounded regular or eccentric nuclei. Epithelial cells from larger bile ducts may be found in large sheets showing a honeycomb pattern [8], with a focal gland like arrangement. Nuclei have finely granular pattern, inconspicuous nucleoli with high N/C Ratio[8]

Sinusoid endothelial cells :

Larger size than bile duct epithelial cells but smaller than hepatocytes. They are polygonal with vesicular nuclei containing prominent nucleoli. They are rarely seen[8].

Kupffer's cell:

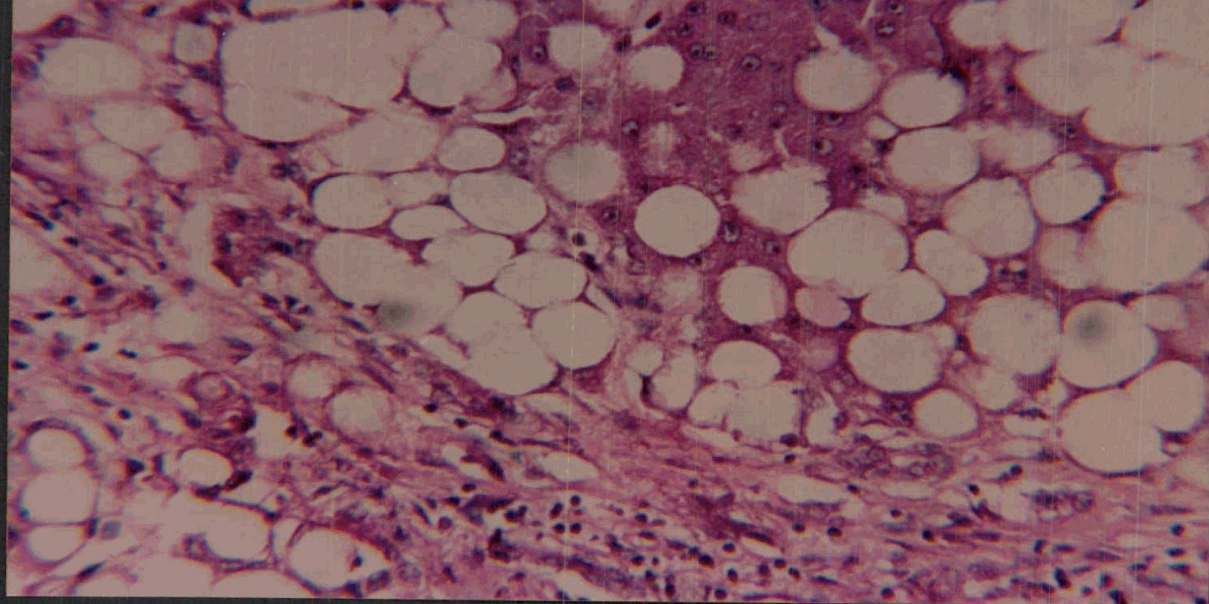
Rarely seen.

They are of various sizes [8] They are seen singly or in sheets of sinusoidal endothelial cells, Comma shaped with single ovoid nucleus.[8]

BENIGN ASPIRATES

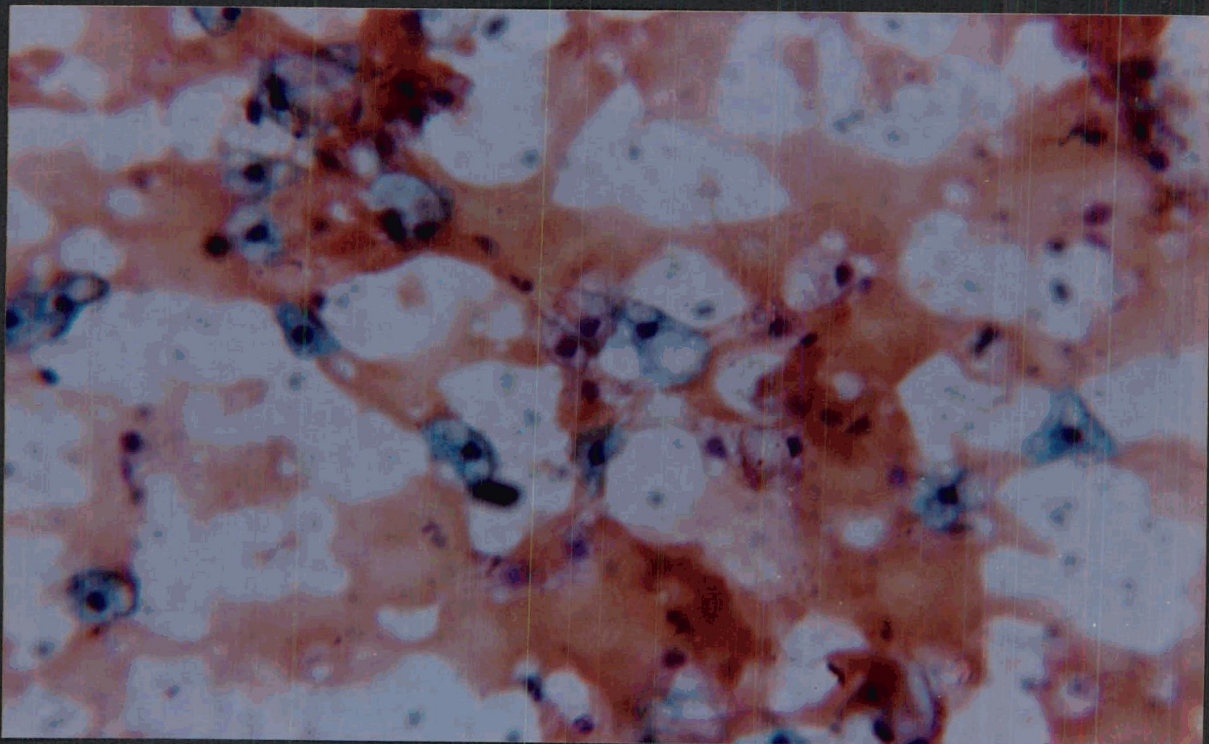
1> Inflammatory lesions

In such cases either purulent or anchovy sauce like material is aspirated and background is full of inflammatory cells, like polymorpho nuclear cells, lymphocytes etc... with necrotic material and absence of tumor cells[1, 8].



Section showing Fatty Liver

- H & E * 192



Smear showing Fatty Liver

- PAP * 300

2> Cystic lesions

Aspiration of a clear fluid confirms the diagnosis grossly and a membrane filter preparation may reveal of only a few foam cells or fragments or epithelial cells from the lining of the cyst [8].

3> Fatty liver[8]

Here the most characteristic finding is the presence of intracytoplasmic vacuoles.

4> Cirrhosis

Smear shows increased cellularity [5] with decreased cohesion. Single files and groupings of hepatocytes are seen[5,10]

Cells show anisocytosis and anisonucleosis with paler cytoplasm. Cell membrane is indistinct and may show intracytoplasmic vacuoles[6,8,10]. Acidophilic and alcoholic hyaline globules are also seen, however difficult to identify in cytologic preparation [10]. Nuclei are eccentrically placed with finely granular chromatin and occasional irregularity of nuclear membrane. Multiple irregular nucleoli as well as prominent round single nucleoli are also seen.

N/C Ratio is not constant from cell to cell.

Few hepatocytic naked nuclei are seen[17]

Bile duct epithelial cells, kupffer cells, fibrous tissue and lymphocytes increase in the cases of cirrhosis [10].

Liver cell dysplasia [LCD] -[Regenerative & Degenerative hepatocytes.]

Material from regenerated hepatic nodule containing metastasis or cirrhosis show large bizarre looking hepatocytes with enlarge single or double nuclei showing intranuclear cytoplasmic invaginations [8].

Cells may display anisocytosis, nuclear pattern is finely granular [8]

Dysplastic hepatocytes are usually seen in apparently benign liver parenchyma around a HCC [8] in liver of patients with Hbs Ag positive or cirrhosis [8].

They are either singly or in clusters and pleomorphic [8]. Large nuclei and show irregular clumps of chromatin and prominent nucleoli [8]

LCD, such as cirrhosis, have a tendency to progress towards cancer and to be occasionally associated with HCC [25]

5> Hepatitis [20]

Cytology of acute hepatitis show increased number of mononuclear cell infiltration, kupffer cells which often contained phagocytosed pigments. Bile plugs in varying number were always found in the canaliculi and often in the hepatocytes. The glandular epithelial cells show pleomorphism with numerous bi or multinuclear cells and some times mitotic figures are seen. Often pronounced anisochromasia are seen. The absence of fatty vacuolation is seen.

6> Liver Cell Adenoma [LCA]

More commonly seen in female patients specially taking oral contraceptives for long periods [> 5 years] and that regression of lesion occur in some cases after stopage of the medication.

Cells are normal or enlarge in size arranged in clusters. Cytoplasm is either clear or vacuolated reflecting their high glycogen or fat content. Single or double nuclei with prominent nucleoli, areas of haemorrhage or necrosis may be noted [8].

7> Focal Nodular Hyperplasia [8] FNH

It is an incidental finding on laprotomy and is diagnosed on histopathologically. These lesions are commonly seen in female patients, with age incidence ranging from 14 months to 74 years. These lesions are difficult to diagnose on FNAC where only normal hepatocytes are seen.

8> Bile Duct Adenoma [8] BDA

Incidental finding at laprotomy or autopsy. Their FNAC has not been documented in the literature.

9> Haemangioma

It shows blood and few irregular sheets of endothelial cells with slight anisokaryosis on FNAC and hence their diagnosis is confirmed with radiological correlation [8]

10> Dysplastic Nodules [14,25]

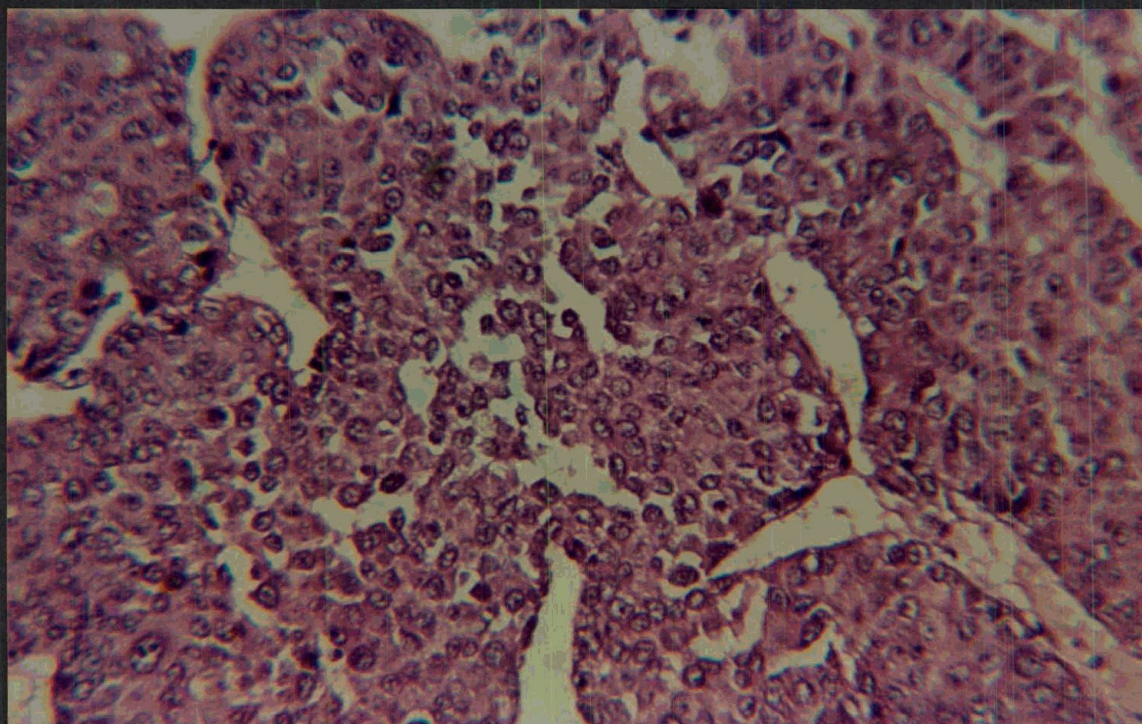
These lesions manifest as minute nodule in cirrhotic livers. They occur due to repeated episodes of necrosis and regeneration that cause alterations in the microenvironment eg. fibrosis with obliteration of sinusoids, which are presumed to be important in establishing a cell line capable of proliferating in a self perpetuating manner. The unusual nodule then established can be referred to as a "dysplastic nodule"

Histologically they reveal basophilic hepatic cord in a focal areas of an otherwise simple hyperplastic nodule [14]

Cytologically numerous atypism and pleomorphism seen individual hepatocytes are small and the nuclei are uniform in size and shape. Following are the nuclear abnormalities more seen in the dysplastic nodule compare to hyperplastic nodule.

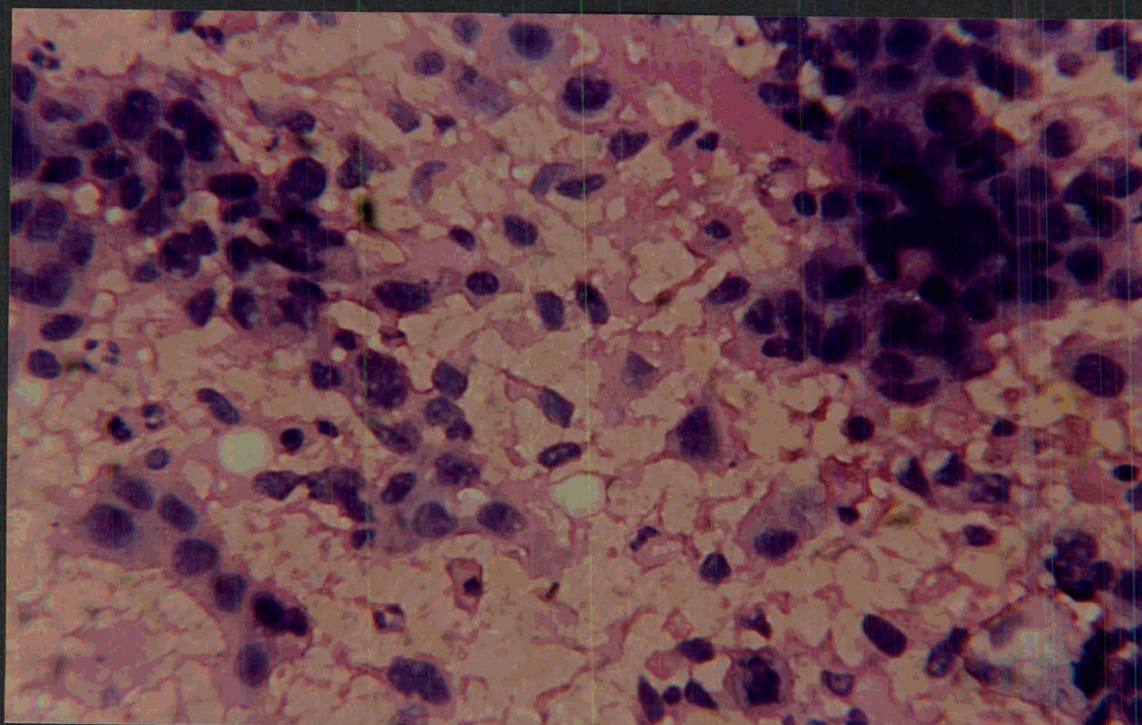
- a) Relatively large nucleolus in comparison with cellular and nuclear size.
- b) Thickened nuclear membrane.
- c) Indentation, convolution and other irregularities in the nuclear outline.

Nonetheless, regenerative, degenerative & neoplastic hepatocytes are connected by a subtle tie. It is hence some times difficult to determine the morphologic steps of the malignant transformation.



Section showing WD HCC

- H & E * 192



Smear showing WD HCC

- H & E * 300

MALIGNANT LIVER ASPIRATE

1> Primary tumors

2> Metastatic tumors

a) Hepatocellular carcinoma

It is a leading cause of death after infancy and childhood in China and South East Asia and most of the tropical Africa. The tumor is relatively rare in Europe and North America [8].

90 % of the HCC cases are associated with, and probably caused by persisting infection with hepatitis B Virus. In general, it is commonly associated with alcoholic micronodular cirrhosis in Europe and North America, but more often with macronodular cirrhosis of viral or toxic origin in South East Asia and Africa.

Criteria for HCC to diagnose on smear :

Very cellular smear with tissue fragments [7]. Four patterns seen [8,12]

1> Trabecular :

Long and broad trabeculae often anastomosing and external surface is frequently enveloped by a layer of flattened endothelial cells.

2> Acinar or tubular pattern :

Acinar structures are often small with inspissated bile could be seen filling the acinar lumina. This is an important feature to differentiate acinar type of HCC from cholangiocarcinoma and adenocarcinoma.

3>Anaplastic variant :

Rarely seen, with highly pleomorphic and spindle shaped cells.

4>A dispersed cell pattern :

Usually seen in combination with other cell patterns. As a rule more undifferentiated tumors more single cells are observed.

Cellular features :

Cells show abundant eosinophilic cytoplasm (PAP) or bluish cytoplasm (MGG). They often contain vacuolation. Intracytoplasmic inclusions are seen.

Nuclei are round to oval and often centrally located with thick nuclear membrane and prominent eosinophilic nucleolus [4,12] they contain coarsely or finely granular chromatin with occasional intranuclear cytoplasmic inclusions (i.e. Nuclear holes) [1,17]

N / C Ratio is increased.

Bile duct epithelial cells not seen. Bile plugs seen between tumor cells which is pathognomonic features of HCC [8].

HCC are of four types according to cytological features.

1> Well differentiated HCC {WD HCC}

2> Moderately differentiated HCC {MD HCC}

3> Poorly differentiated HCC {PD HCC}

4> Undetermined

Naked nuclei in liver aspirates :

They are seen in malignant aspirates especially HCC, Cholangiocarcinoma and metastatic lesions [15,17]

In HCC they are seen in background and numerous in WD HCC and less WD HCC.

Their shape is roundish, flower like or polymorphic, later two are seen in more commonly in PD HCC. They reflect the nuclear features of the adjacent malignant hepatocytes but with more distinctive atypia. They have single centrally placed nucleolus[15,17]

Cholangiocarcinoma and metastatic carcinoma show naked nuclei but hepatocytic naked nucleoli are not seen [15,17]

Importance [15,17]

1>Regenerative and degenerative liver cells do not show this naked hepatic nuclei and hence helps in to differentiate from HCC. If at all they are seen in later conditions - lack signs of atypia

2>In WD HCC naked nuclei do not show significant atypia so helpful differentiate from PD HCC

In case of breast aspiration, stripped nuclei indicate benign aspiration

Problems while facing diagnosis of HCC

1>PD HCC are difficult to distinguish from large cell undifferentiated carcinoma of the lung and metastatic adeno carcinoma. But HCC shows bile plugs, immunoperoxidase staining for AFP and naphthylaminidase staining for bile canaliculi [10]. It does not show mucin production.

2>WD HCC may be difficult to distinguish from benign representative nodules or adenocarcinoma because of nuclear enlargement, anisokaryosis, macronuclei and dissociation may be very prominent in regenerative nodules [10]

In malignancy cellular smear with glandular and trabecular pattern, irregular distribution of nuclear chromatin and absence of bile duct epithelial cell is noted

Fibrolamellar HCC [8]

Seen in young patients where the tumor cells are polygonal with deeply eosinophilic cytoplasm and are surrounded by lamellar fibrosis. This type of HCC is usually localised, permitting complete resection and has a more favourable prognosis [8].

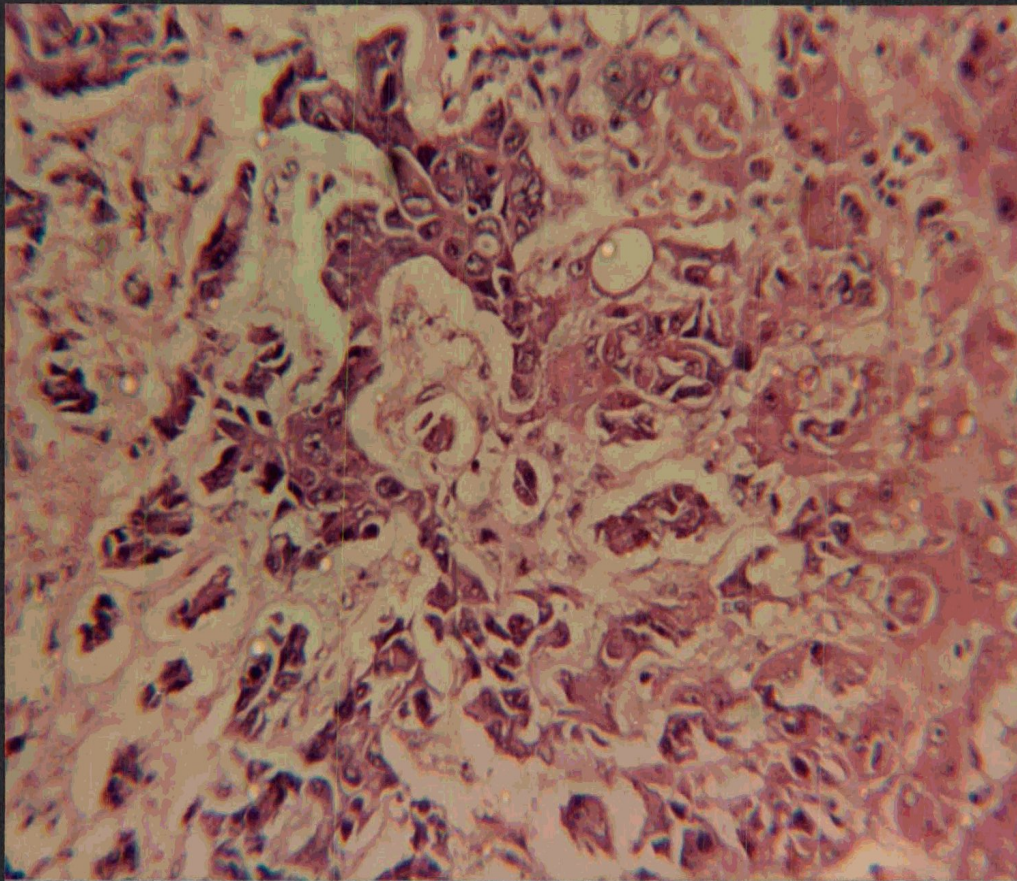
They do not produce bile and do not contain AFP.

Primary Neuroendocrine tumors of liver [22]

Primack et. al, described HCC associated with the carcinoid syndrome. Liver apudomas are described as having abundant endocrine granules.

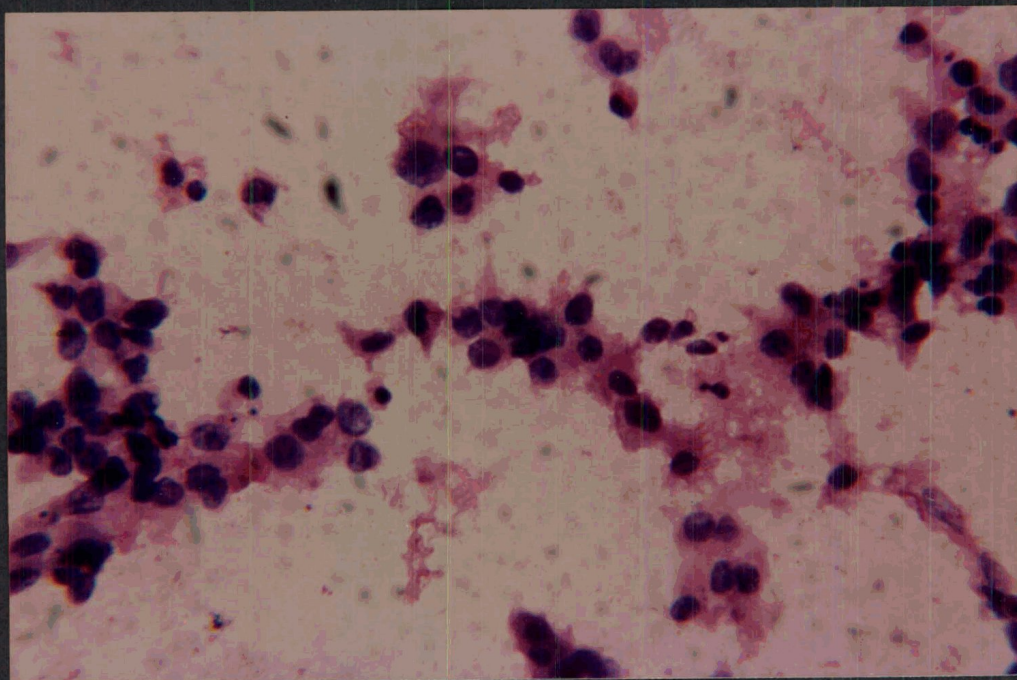
Relationship of HCC and AFP [14]

The production of AFP can be demonstrated by immunohistochemistry in malignant hepatocytes even in those patients who have only a slight amount of serum AFP. They are generally lacking in both WD HCC and Anaplastic HCC. There is an inverse relation between number of albumin and AFP positive cells

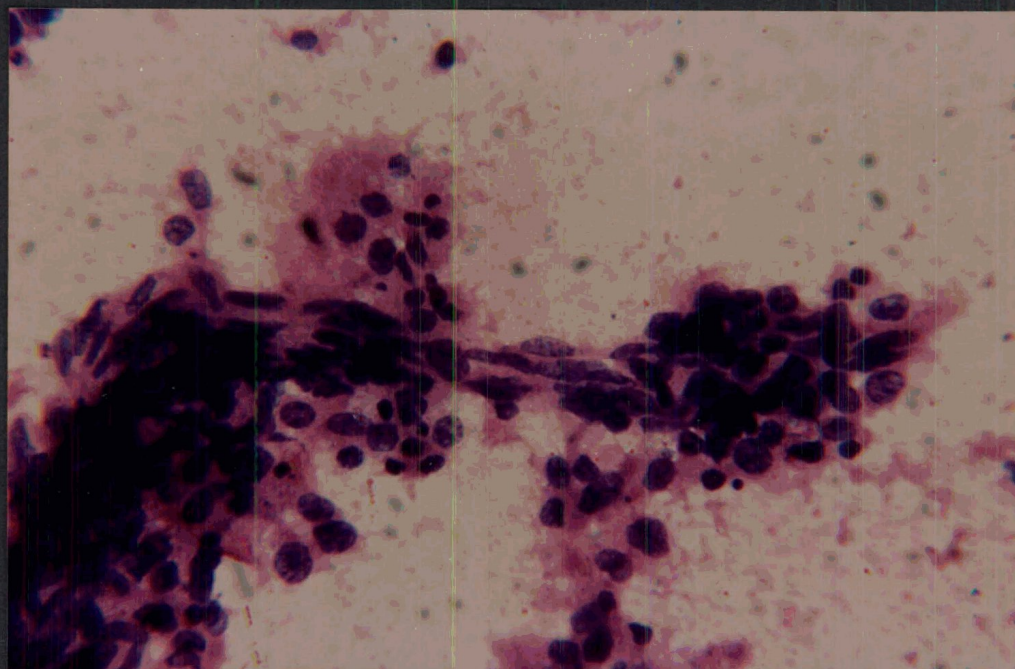


Section showing mets. from Breast

- H & E * 192



Smear showing mets. from Breast - H & E * 300



Smear showing Endothelial cells - H & E * 300

suggesting that the tumor cells are capable of synthesizing only one of the two proteins.

Normal hepatocytes and cirrhotic cells do not show AFP positivity.

Cytological diagnosis of HCC should be made only when all of the following criterias are met [5]

- 1>Serum AFP level should be ascertained when HCC is being considered.
- 2>Aspiration must be cellular and must containe tissue fragments.
- 3>Atypical cell population should show uniformity. A mixed population of hepatocytes [atypical cells intermingled with bland cells] favours the diagnosis of a dysplastic but not a neoplastic process.
- 4>The presence of multinuclear tumor giant cells is usually considered to be nearly pathognomonic of Hepatoma.

CHOLANGIOCARCINOMA :

It is an intrahepatic malignant tumor compsed of cells resembling those of bile duct epithelium [8]. Usually it is solitary [8].

Histologically the pattern is usually tubular rarely papillary or solid and may be mixed [8].

Cells are less cohesive & single cells are often seen. Nuclear crowding is noted but nuclear enlargement and pleomorphism are not prominent[10].

Sr.No.	Features	WD. HCC	MD. HCC	PD. HCC	Undetermined
1.	Pattern .	Trabecular & glandular (Micro)	Trabecular & glandular (Micro)	Cobblestone	Undeveloped
2.	Cells .	Less Cohesive & form tight clusters but do not overlap	Less Cohesive & with overlapping	Tumor cells in clusters in the centre & overlap each other but at periphery cellular cohe- sion is lost.	Less of cellula Cohesion.
3.	Nucleus .	Round,centrally located & often multinucleated	Round to oval, peripherally located & often multinucleated.	Varying	Large pleomorp- hic & spindle shaped
4.	N/C Ratio.	Slightly Increased	Moderately increased	Highly increased	Highly increased.
5.	Nucleoli.	Prominent	Round,Centrally, Prominent	Small irre- gular multi- nucleoli.	Giant Nucleoli
6.	Nuclear Membrane.	Thin	Thin	Very thin	--
7.	Chromatin.	Coarse granular unevenly distributed	Coarse granular unevenly distributed	Abundant finely g granular.	--
8.	Cytoplasm.	Granular, yellow, brown granular, often with intra- cytoplasmic granules abundant	Less abundant. Few yellow brown granules	Scanty, pale, ill defined Margin.No granules	Abundant & contain fine fibrous structures.
9.	Similar to normal Hepatocytes.	Similar	less Similar	few	undetermined

Problems in diagnosing Cholangiocarcinoma :

1>. They often show abundant desmoplastic stroma,hence difficult to prove[10].

2>. It has to be differenciaded from.....

a). HCC

Cholangiocarcinoma shows normal bile duct epithelial cells and no hepatocytes on smears.

b). Non neoplastic lesions of liver

Cholangiocarcinoma shows large number and size of sheets.

c). Metastatic Adenocarcinoma :

It is often positive for AFP [10]. After staining with amino acid naphthylamidase,it will show presence of bile canaliculi on the smear of cholangiocarcinoma which is absent in adenocarcinoma[12].

HEPATOBLASTOMA [29].:

Cytological smears are highly cellular and are arranged in acinar or in small groups often with bile plug.

Cells are oval with abundant cytoplasm with often fat vacuoles.

Centrally placed nuclei with small but distinct nucleoli.

No pleomorphism or giant cells seen.

Smears also show haemorrhagic background where there is a large number of normoblasts, myelocytes,metamyelocytes and occasional megakaryocytes.

Histologically :

Embryonal and

Fetal tpe.

Former shows small fusiform cells with hyperchromatic nuclei little cytoplasm. Arranged in ribbon or rosettes. Fetal one shows large cells with more cytoplasm containing glycogen and fat. Arranged in trabecular with canaliculi and sinusoids.

METASTATIC CARCINOMAS :[8].

Metastatic carcinomas occur more frequently in the liver than in any other organ. Most metastases in the liver arise from abdominal organs drained by the portal vein, primary cancer of the gall bladder, extrahepatic bile ducts pancreas and stomach may invade the liver by direct extension. Cancer arising in other organ metastatize to the liver via hepatic artery or by direct invasion from peritoneal implantation.

One of the most chalanging problem in the practice of aspiration biopsy cytology is to suggest the location of an occult primary tumor that has given rise to a liver metastasis.

In a search for the primary tumor morphologic evaluation of hepatic material obtained by FNAB or tissue biopsy is always one of the first important step of laboratory investigation.

The first step is to identify and classify tuomr cells in a broad catagories of adenocarcinoma, squamous cell carcinoma , anaplastic carcinoma, melanoma, lymphoma, carcinoid tumor or sarcoma . Once the tumor cells have been identified, the search for the primary cancer can be concentrated on the organ or the

system where that type of cancer is more commonly associated with liver metastasis. According to Douglas, the most common site of occult cancer with hepatic metastasis is the lung followed by pancreas, colon and stomach. Almost all primary cancers, except those arising from the brain, can metastasise to the liver. Metastatic malignancies can be classified into four categories.....

1>Carcinoma .

2>Sarcoma .

3>Lymphoma .

4>Melanoma .

Cytological features of these malignancies are as follows. [24]

	Carcinoma	Sarcoma	Lymphoma	Melanoma
COHESION OF CELLS	More	Many single cells	All single usually	All single & Binucleated
MOULDING	Conspicuous	No	No	No with well defined borders
PLEOMORPHISM	more in size than shape	Spindly	Round to oval with mild pleomorphism.	Round or oval
OTHERS	Sq. or Glan-dular diff.	Extracellu-lar material on MGG	Small glob-ular bodies in backgr-ound.	Eccentric nuclei with prominent nucleoli & intranuclear vacuoles.

Cytological Features Of Different Varieties of Carcinoma ;
Adenocarcinoma ;[24].

Usually cells are smaller than squamous cells and may appear columnar, cuboidal or round to oval. They may be arranged in flat, monolayered sheets pallisade forms, acinar, papillary fragments, linear forms or cell ball cluster.

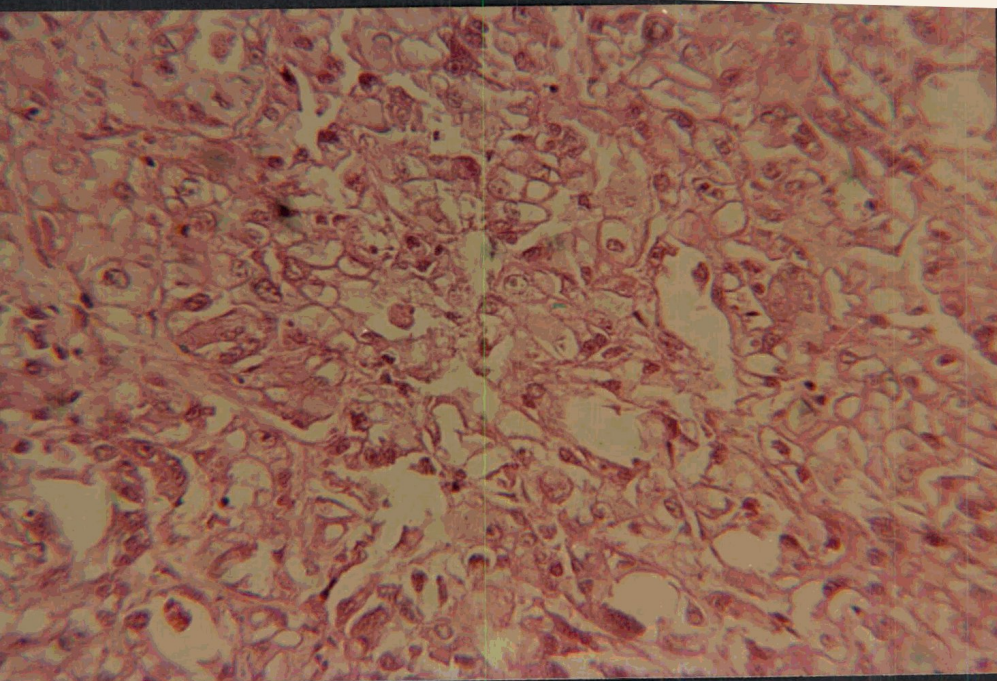
Cytoplasm may be vacuolated or granular and weakly cyanophilic.

Nucleus may be round or oval, central or eccentric either non chromatic or slightly chromatic. Chromatin material is finely granular and often irregularly distributed. Large centrally located nucleoli often seen. Altered N/C ratio is noted.

Some times products of secretion such as mucin may be evident. Psammoma bodies are seen especially in papillary tumors from ovary and thyroid. Tumor cell necrosis is often seen.

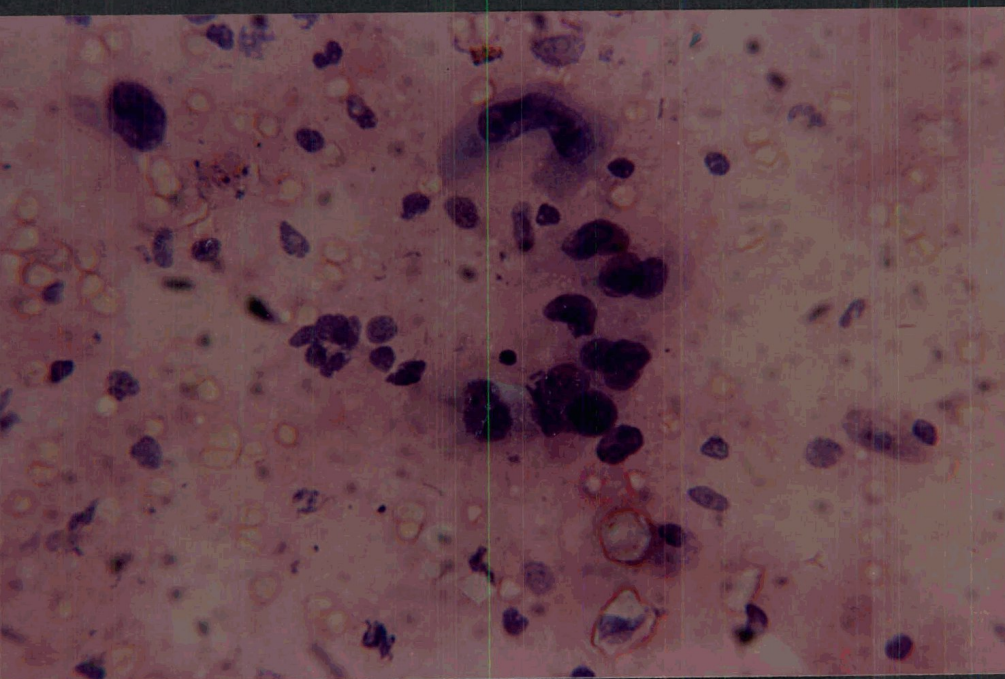
The aspiration cytology of metastatic WD colonic adenocarcinoma displays a rather distinctive pattern. The cancer cells are columnar and occur in groups or in syncytium like clusters forming gland like foci. Tumor cells nuclei at the free border of the clustered cells may be arranged in vaguely pallisade like pattern.[8].

FNAC of liver metastasis of signet ring carcinoma of stomach, GB & colon may yield single mucus secretory cancer cells with large intracytoplasmic vacuoles.[8].



ion showing mets. from Prostate

- H & E * 192



ar showing mets. from Prostate

- H & E * 300

PD.adenocarcinoma yielded glandular cancer cells of columnar configuration[8].

Mets in liver of Papillary adenocarcinoma of region near the ampulla of Vater show irregular papilla like clusters of malignant glandular cells with regular nuclei and scanty cytoplasm[8].

Adeno Ca. of Pancreas[10].

Large columnar cells with elongated nuclei & focal palisading. Arranged in microglandular pattern. Squamous differentiation is often noted and may be prominent. Anaplastic Ca. have large pleomorphic bizarre giant cells.

Adeno. Ca. of Prostate[8].

Small cells, regular, in sheets with a focal gland like pattern.

Adeno. Ca. of Ovary :

Medium sized cells [2], singly or in papillary, glandular or solid aggregates with scanty cytoplasm & occasional vacuolation.

Pseudomembranes noted.[10].

Adeno. Ca. of Breast :

Small to medium sized cells[2], cords or glandular clusters. Individual scattered malignant cells seen in the background.[3]. No stripped nuclei[20].

Adeno. Ca. of Uterus[10,24] :

Small cells in glandular and cribriform pattern.

Adeno.Ca. of Lung[10].:

Medium to large size columnar cells, seen in flat sheets, rosettes or acinic in clusters or balls. Abundant cytoplasm with round to oval eccentric nuclei solitary large nucleoli. Mucin secretion is also seen.

Squamous cell Ca.[24].:

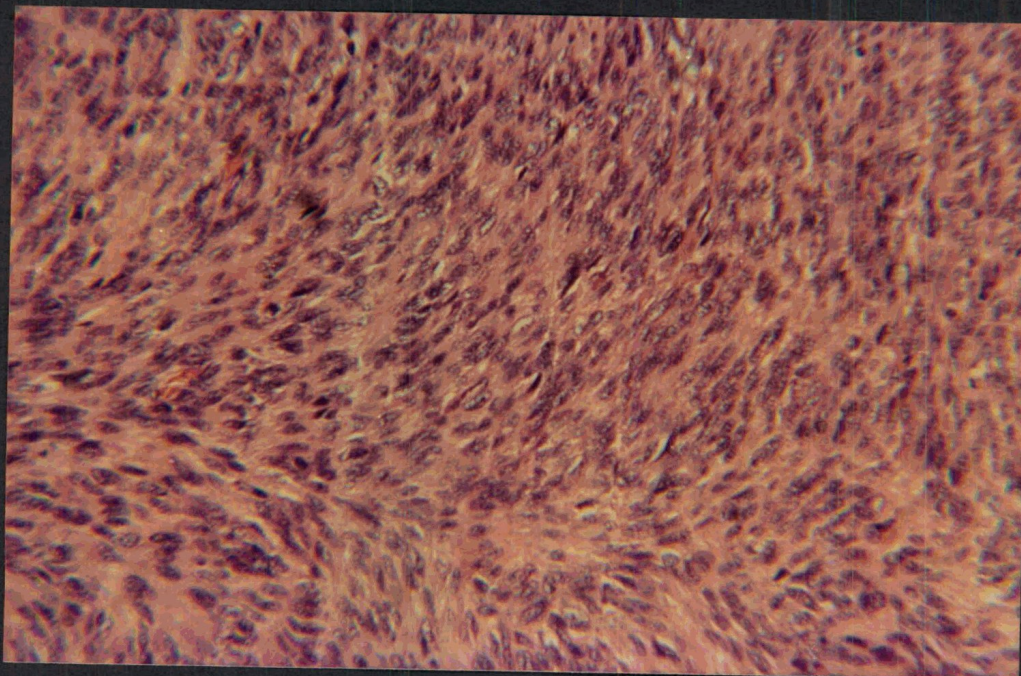
Moderately larger cells either in sheets or syncytial group. Homogenous cytoplasm [Cyanophilic or Eosinophilic-PAP, dense & pure blue-MGG].[10]. Pyknotic nuclei with large prominent irregular shaped nucleoli and with irregular coarse granular chromatin. Increased N/C ratio. Necrotic tumor cell with keratin often seen.

In WD.-more single cells with many pyknotic nuclei with low N/C ratio. Cytoplasm tends to stain eosinophilic keratin pearls may be seen.

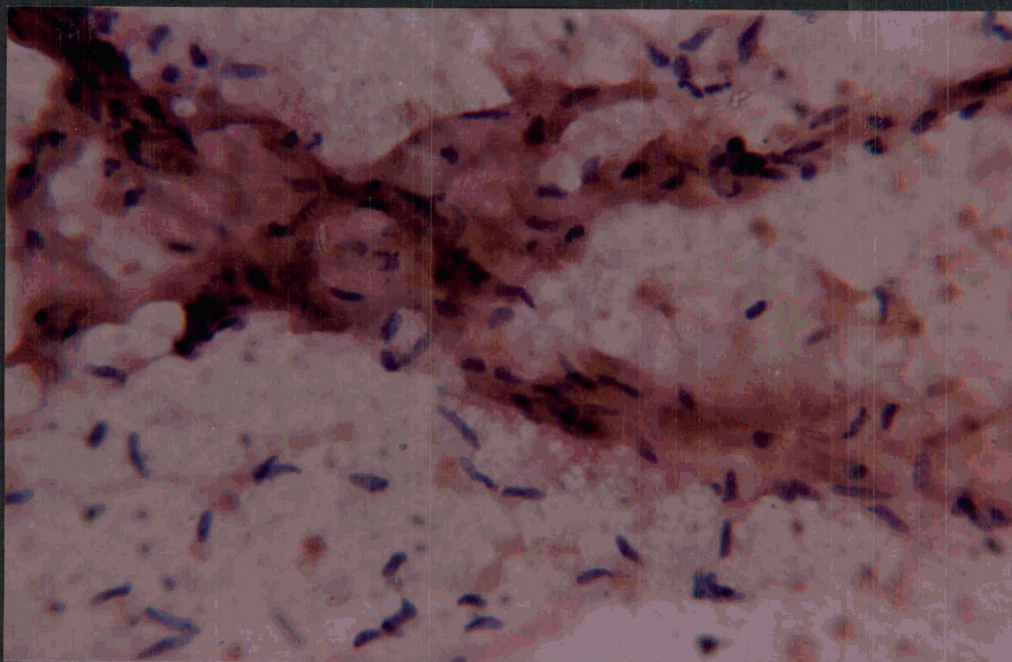
In PD.-cells seen in sheets with distinct cell borders. Homogenous cytoplasm. Keratinisation of the cells inconspicuous or absent. Nuclei rarely pyknotic & irregularly coarsely granular chromatin with prominent nucleoli.

Small & Large cell Ca. [10]:

In small ca.- small to medium cells either in loose clusters or tight small group with little or no cytoplasm & irregular nuclei. Nuclear moulding is seen. They coarsely granular chromatin with small nucleoli.



Section showing mets. from Intestinal
soft tissue Tumor (Leiomyosarcoma) - H & E * 192



Smear showing mets. from Intestinal
soft tissue Tumor (Leiomyosarcoma) - PAP * 300

In Large cell Ca.- large pleomorphic cells with abundant but fragile cytoplasm. Tumor giant cells seen & nucleus contain macronucleoli.[10]. The most likely primary site for large cell carcinoma is Lung[10].

SARCOMA [24].:

1> Round cells or Spindle cells.

e.g; Ewings sarcoma, Angio sarcoma & Synovial sarcoma.

2> Spindle cells.

e.g; Leiomyosarcoma, Fibrosarcoma, Malignant schwannoma.

3> MFH, Liposarcoma and Osteogenic sarcoma have pleomorphic cells.

4>Rhabdomyosarcoma have round cells,spindly and pleomorphic cells.They show rhabdomyoblasts which have eccentric nuclei and tail of dense eosinophilic cytoplasm.

LYMPHOMAS [24]:

Noncleaved type of lymphoma shows monotonous population of single lymphoid cells which are larger than their counter part. Uniform rounded slightly larger nucleus. Chromatin is evenly distributed and not dense compared to that normal lymphocytes.

Small type: Nuclei are larger, variable in size & show clearing. They have tendency to form clusters which lead to some moulding of nuclei.

Small & Large type of lymphomas :

In addition to predominantly small cell, a small proportion of large centroblast are noted, having large vesicular nuclei of irregular shape which are sometimes distinctly cleaved. Nucleoli are present & often located near nuclear membrane. Small amount of cytoplasm is present. When the centroblast exceed 25% of the mixed population, the lymphoma is classified as large cell type.

Immunoblastic [Large Cell- Non Centroblastic] lymphoma:

Pleomorphic population with prominent large blast cells. They have large nuclei of variable size and shaped with centrally located prominent nucleoli. They have coarse granular chromatin. Some of them show Plasmacytoid differentiation.

Malignant Plasmacytoma :

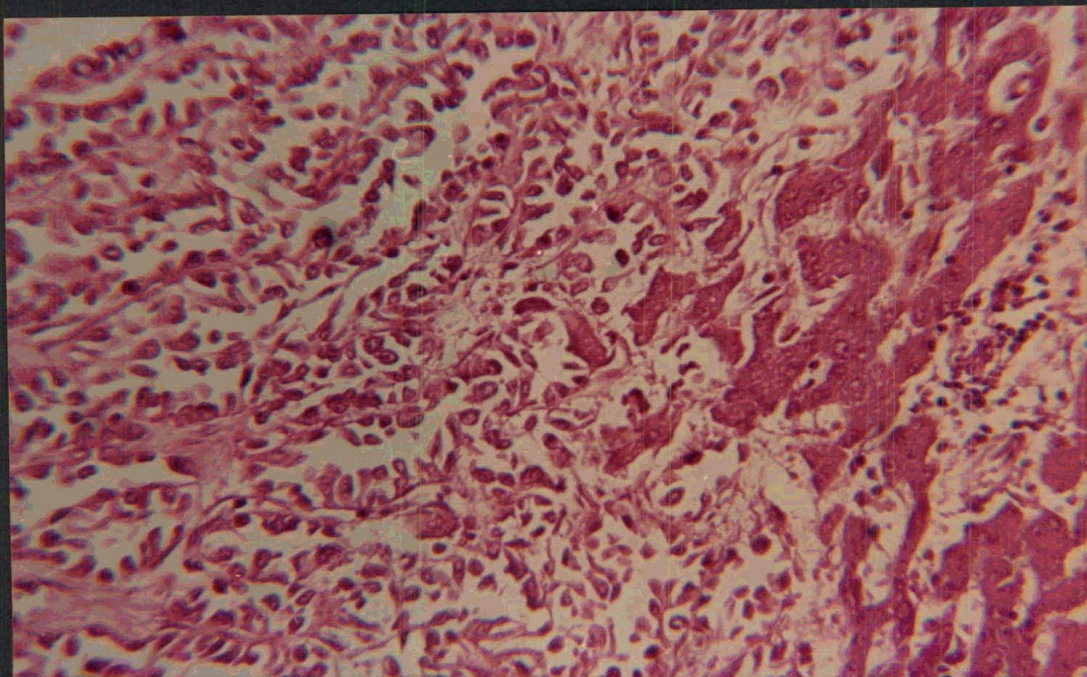
-shows moderately pleomorphic plasma cells. Abnormal plasma cells are oval with a round eccentric nuclei. Cytoplasm is cyanophilic with a clear zone adjacent to the one side of nucleus. Some of the chromatin tends to be peripherally clumped giving it a cork wheel appearance.

Lymphoblastic lymphoma :

Similar cytological features to that of centocytic type except for prominent nucleoli & nuclear chromatin.

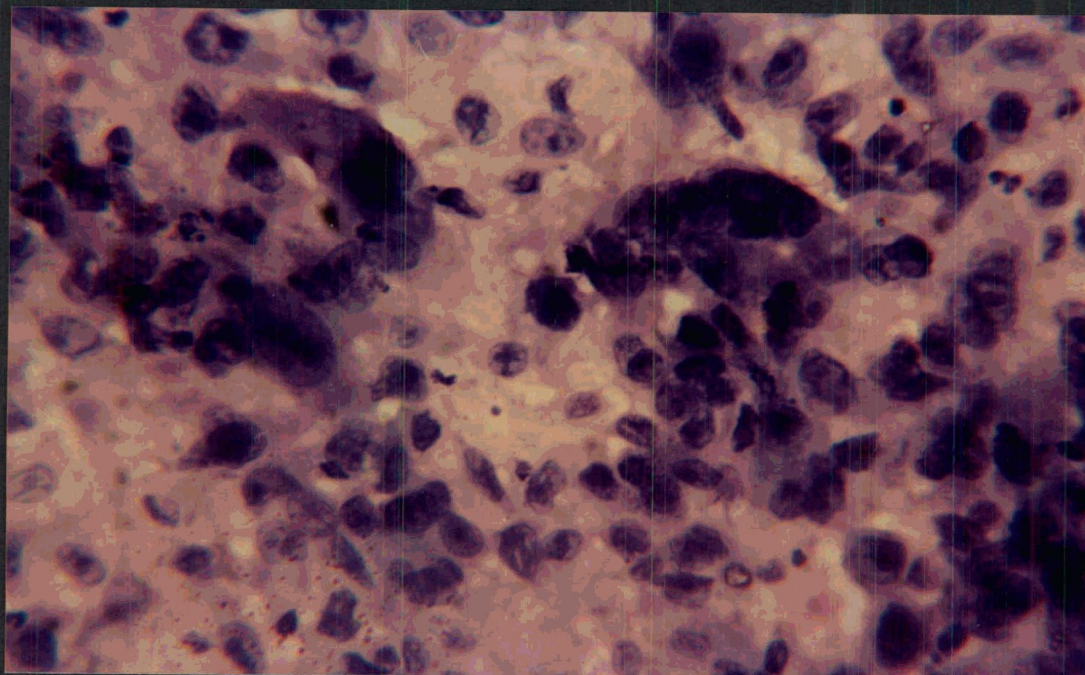
Burkitt's lymphoma :

Cells are primitive with round to oval nuclei and amphophilic cytoplasm. Nuclei contain prominent nuclear membrane and coarsely



Section showinng mets. from RCC

- H & E * 192



Smear showinng mets. from RCC

- H & E * 300

clumped chromatin. They contain 2 to 5 prominent nucleoli.

Hodgkins lymphoma :

Classical RS cells seen. Primary lymphoma is very rare but cytologic differentiation between primary secondary lymphomas of the liver is impossible.

MELANOMA :

Cells show cyanophilic cytoplasm, no broad trabeculae. Diagnostic is melanin pigment.[12].

OTHERS :

1> Carcinoid - metastatic [12] :

-small tumor cells ; no macronuclei. Cytoplasm - finely granular & basophilic . Elevated 5HIAA (5- hydroxy- indole acetic acid).& serotonin are seen with metastatic carcinoid patient. Grimelius staining of the cells revealed intracytoplasmic brown black granules

2> Metastatic Renal Cell Carcinoma [3,8,10,20] :

-cytology shows round with eccentrically placed nuclei with macronucleoli. Abundant basophilic cytoplasm with granules or lipid droplets. The cells are single, in solid alveolar pattern or papillary or form irregular sheets with strong cellular cohesiveness..

3> Mets of TCC of Bladder :

Cells are singly or in loose clusters. Dense cytoplasm with eccentrically placed hyperchromatic nuclei. They have tendency to squamous differentiation, some of them show spindle cell pattern.

4>Mets of Endodermal sinus tumor [30] :

Cells show indistinct cell borders. They have tendency to form aggregates with microglandular pattern .Large vesicular pleomorphic nuclei & vacuolated cytoplasm. Large eosinophilic nucleoli with coarse but irregularly distributed chromatin.

Often show small spindle cells representating Primitive mesenchymal tissue.

MATERIALS AND METHODS

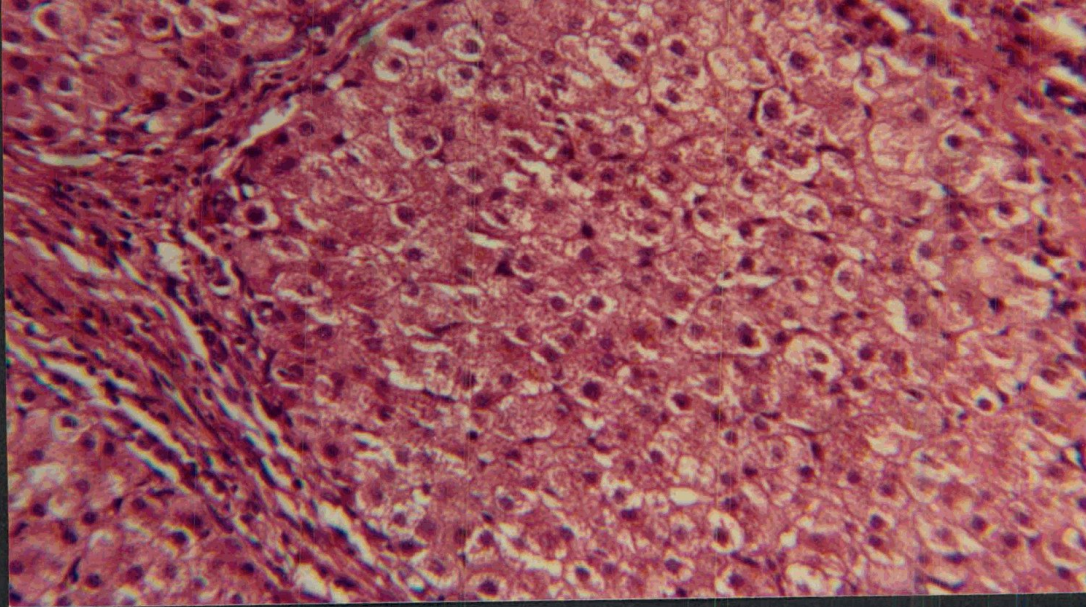
The present study of FNAC of liver includes 45 cases, out of which 42 cases were of post mortem and 3 cases were of surgical.

In all the PM cases where liver was diffusely involved the Aspiration was done from right lobe & where the lesion was localised then it was carried out from the site of lesion. The FNAC was done when the organ was in situ.

Out of 3 surgical cases , in 2 cases the FNAC was done in surgical histopathological department from the hepatectomy specimen while in 1 case the FNAC was done in ward with biopsy sent afterwards.

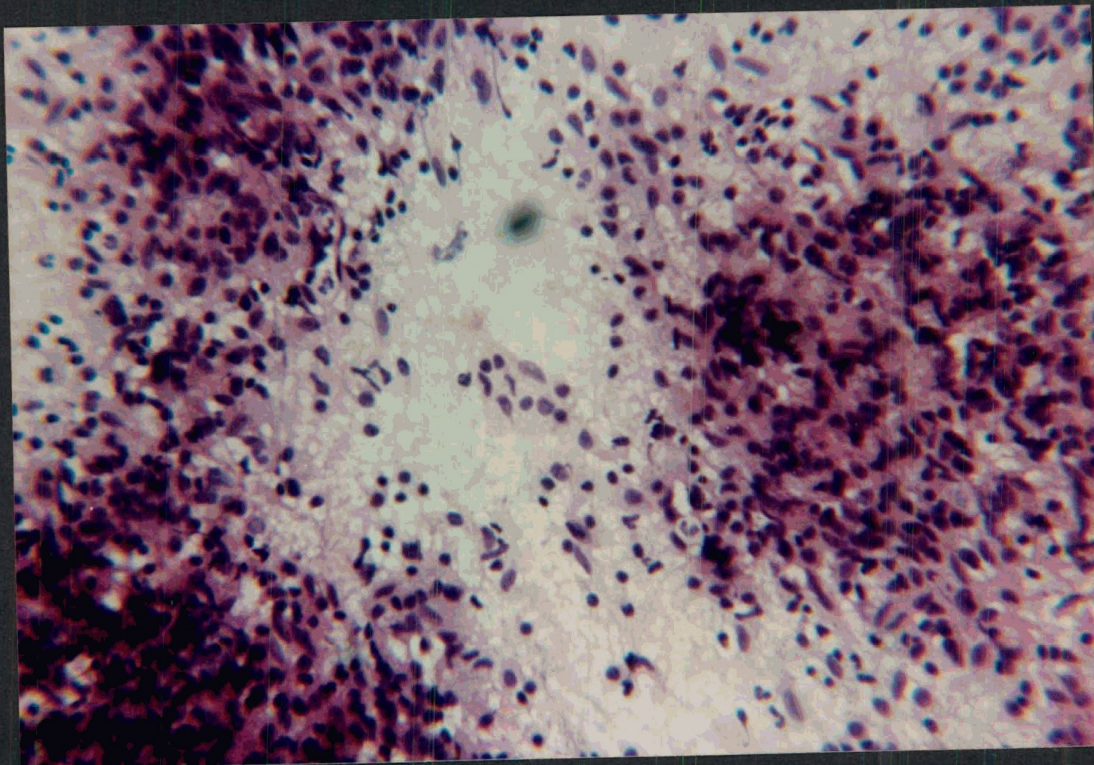
In our study the total number of males were 34 & females were 11 .The maximum number of patient in our study were in the age group of 41 to 50 years of age.

The aspiration was carried out in PM room with shortest time interval after death was 3 hours & maximum was 24 hours after death.



Section showing Cirrhosis

- H & E * 192



Smear showing Fibrous tissue & Kupffer
cells - Cirrhosis

- H & E * 192

Out of 45 cases we studied ,the 14 cases were diagnosed as Normal aspirate cytologically ,22 as benign while 9 as malignant aspirate. The benign aspirates include-diffuse liver disorders like Infective hepatitis , Fatty change , Cirrhosis ,Malaria and Typhoid cases. The malignant aspirate include the Primary & the Secondary tumors. In case of secondary tumors the origin were from different sites like GB ,Breast ,Prostate Intestinal soft tissue & Kidney .All 45 cases were finally tried to co-rrelate with the Histopathological diagnosis.

Requirements

- :- Disposable Needles 22 to 23 gauge.
- :- Disposable Syringes 10 ml.
- :- Glass Slides.
- :- Fixatives- 95% alcohol and ether in equal combination.

Procedure

In case of diffuse liver disorders the FNAC was done from Right lobe and in localised lesion it was carried out from the site of the lesion .

The syringe is held by piston and the needle tip is pushed into the site of lesion .The plunger is partially retracted causing a negative pressure and then syringe is rotated by a movement of the wrist and gently moved in and out .The cutting edge of the needle tip frees the cells inside the lesion which are sucked into the fine bore of the needle by forward backward and rotating needle

[Jackhammer technique.2].If something tissue or fluid is seen in the body of the syringe i.e. between the bottom of the plunger and needle head, then the plunger is released slowly so that there is no more suction effect. Then the syringe and needle is withdrawn gently and after that the needle is removed from the syringe and air is sucked in the syringe .The needle is fixed again to the syringe and air is blown out on the slides.The material collected on the slide is smeared between opposite surfaces of two slides. This two slides are fixed immediately in the fixative for 30 minutes.

The fixed slides were stained by routine H & E stain , few cases were stained by Papanicoloue stain and in special case like malaria where to differentiate between Iron granules and Hemazoin granules in kupffer cells-Prussion blue staining was done.

First the smears were examined without the knowledge of clinical details but knowing the gross findings in the liver .Again the smears were reviewed with corresponding histology and clinical history.Then the co-rrelation was carried out.

RESULTS

Total number of cases 45.

Post mortem cases 42. (32 males & 10 females)

Surgical cases 03 (2 males & 1 female)

Table I - Age Chart.

Age in years.	0/10	11/20	21/30	31/40	41/50	51/60	61/70	71/80	Total.
Males	-	1	4	9	11	6	2	1	34
Females	2	-	-	2	3	3	1	-	11
Total	2	1	4	11	14	9	3	1	45.

Table II CLINICAL PRESENTATION OF NORMAL CASES

Serial No.	Age & Sex	Presentation
1	16yrs. M	K/C/O CHD Sinus venosus
2	60yrs. F	K/C/O IHD- HT & RF
3	23yrs. M	Jaundice, Unconscious.
4	47 " M	Head Injury,epilepsy with amnesia.
5	34 " M	Oedema feet,abd. distention. oliguria, h/o Malena.
6	55 " M	Chest pain, giddiness,anorexia.
7	60 " F	UC.-(L) -CVA in (Rt.) MCA territory.
8	60 " F	Fracture neck femur (Rt.)
9	45 " M	Oedema feet,Oligouria,Puffiness face,Breathlessness
10	35 " F	Oligouria,Puffiness face,Oedema feet,DOE,Orthopnea.
11	4 1/2 F	Vomitting,Loose motion, Temp.
12	38 " M	K/C/O RHD with MS
13	70 " F	K/C/O HT,DM c/o Dyspnoea
14	30 " M	K/C/O Hanson's disease for ARF management.

TABLE III - LIVER ASPIRATES

Cytological diagnosis	No. of cases
1) Normal aspirate	14
2) Benign aspirate	22
3) Malignant aspirate	09
TOTAL	45

TABLE IV - BENIGN ASPIRATE

Cytological diagnosis	No. of cases
1) Malaria	01
2) Typhoid	01
3) Fatty change	05
4) Cirrhosis	09
5) Cirrhosis + Fatty change	04
6) Miscellaneous	
Necrotic material	01
Fibrocytes	01
TOTAL	22

TABLE V - MALIGNANT ASPIRATE

Cytological diagnosis	No. of cases
1) Primary - HCC	03
- HCC + Cirrhosis	01
2) Metastatic	
- Adeno Ca.- GB	01
- Adeno Ca.- Prostate	01
- Breast	01
- Intestinal soft tissue	01
- RCC - Renal	01
TOTAL	09

TABLE VI - CIRRHOSIS CHART [GROSS]

IAC DIAGNOSIS	No. of Cases	HISTOPATHOLOGICAL DIAGNOSIS		
		C I R R H O S I S		
		Micro.	Macro.	Mixed
CIRRHOSIS	08	---	01	07
CIRRHOSIS + FATTY CHANGE	01	<u>1</u>	---	---

MASTER CHART VI

Cytological Diagnosis	No. of Cases	Histopathological- Diagnosis																
		Normal	C P C	Mala- ria.	Typhoid	Hepatitis	Fatty	Cirrhosis	Fatty Cirrho sis	HCC	HCC+ Cirrh.	Ch.Ca	G.B	Pro- Sta te	Bre- ast	RCC	Intestinal STT	Necrotic
1. Normal Aspirate	14	10	2	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
2. Malaria	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
3. Typhoid	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
4. Necrosis	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
5. Fatty Aspirate	5	-	1	-	-	-	3	-	1	-	-	-	-	-	-	-	-	-
6. Cirrhotic Asp.	9	-	-	-	-	-	-	8	1	-	-	-	-	-	-	-	-	-
7. Fatty+ Cirrhotic Asp.	4	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-
8. H.CC	3	-	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-	-
9. H.C.C. + Cirrhotic Asp.	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
10. Metastatic - GB (Adeno.Ca)	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
- Prostate (Adeno.Ca)	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
- Breast	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
- Intestinal (STT)	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
- Renal	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
11. Miscellaneous - Fibrocytes	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
TOTAL	45	10	3	1	1	1	4	8	6	2	2	1	1	1	1	1	1	1

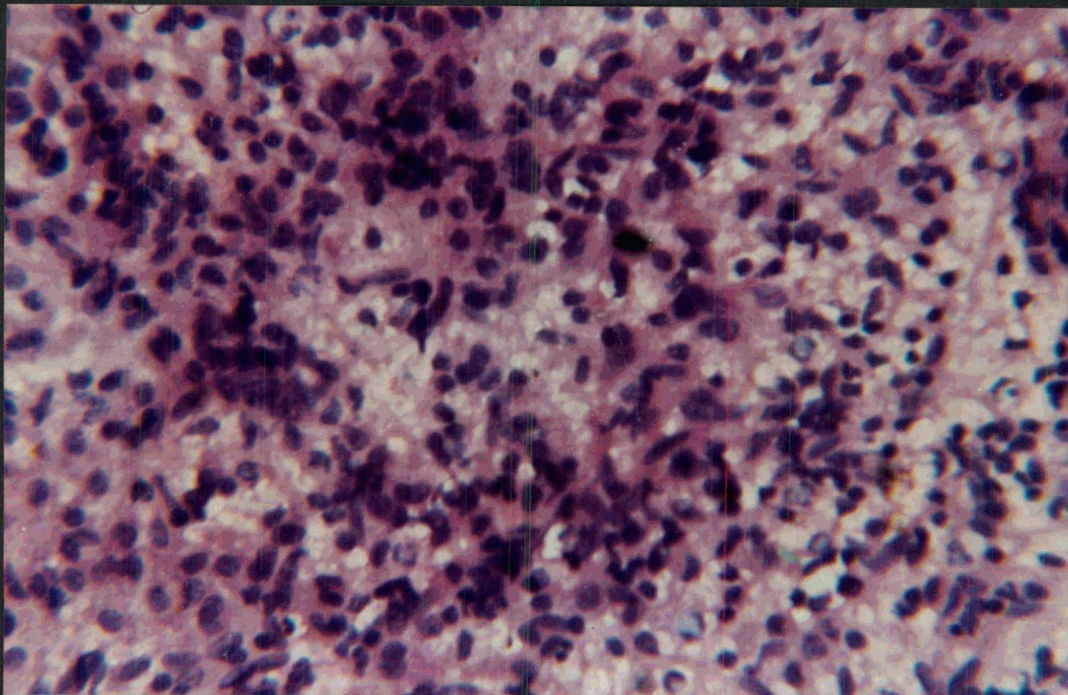
DISCUSSION

1> NORMAL ASPIRATION :

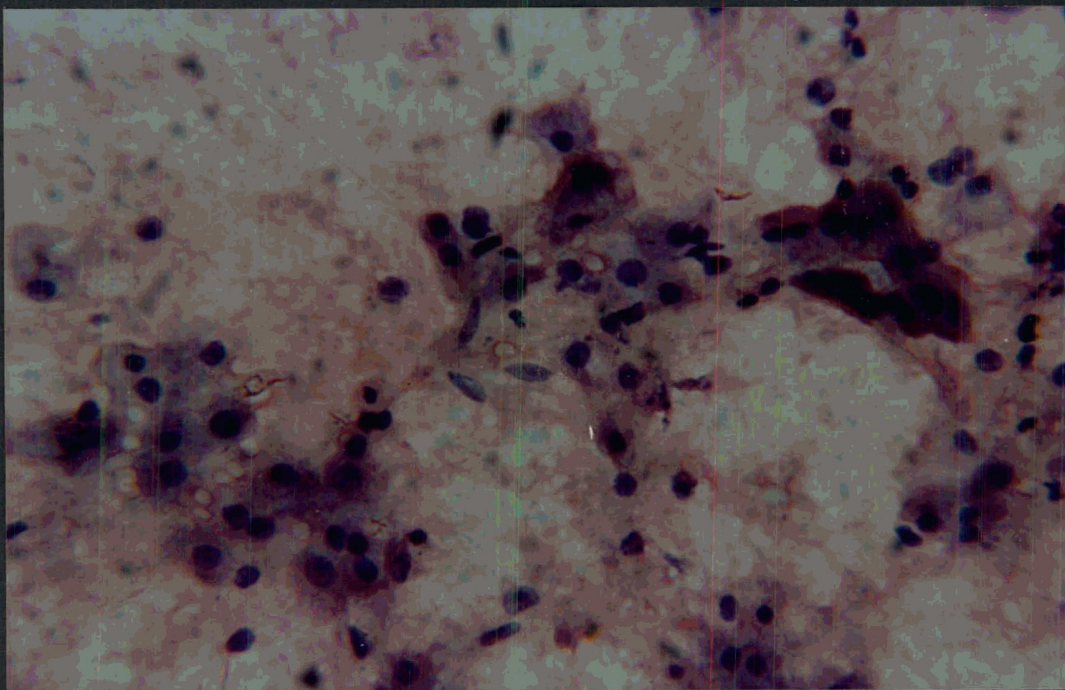
Majority of the cases diagnosed as normal aspirate showed small clusters of benign hepatocytes. They were round to oval or polygonal with abundant granular eosinophilic cytoplasm. They were arranged in groups or singly or in cord like pattern. Nuclei were central, rounded or vesicular and occupy about 1/4 th of the cell with finally distributed granular chromatin with one or two prominent rounded eosinophilic nucleoli. Mitosis not seen. BDE cells and kupffer cells were seen in few cases with rarely fragments of sinusoidal or venous endothelium.

Similar features were also noted by Nguyen G.K. Fine Needle Aspiration Biopsy Cytology of Hepatic Tumors in Adults. PA Part I 321-349/1986[8]

Out of 14 cases diagnosed as normal aspiration on cytology, 10 cases were confirmed by correlating with histopathological diagnosis, while 2 cases were found as CPC, 1 as focal fatty change and 1 as centrilobular necrosis on HP examination. In all of these 4 cases we can say that the FNAC were might not from the exact sites, because the fatty change was focal and not generalised while in other 3 cases the necrosis were centrilobular-patchy. Hence the liver aspirates were not representative [photograph].



Smear showing Fibrous tissue & Kupffer
cells - Cirrhosis - H & E * 300



Smear showing Fibrous tissue & Kupffer
cells - Cirrhosis - H & E * 300

2>FATTY LIVER ASPIRATION

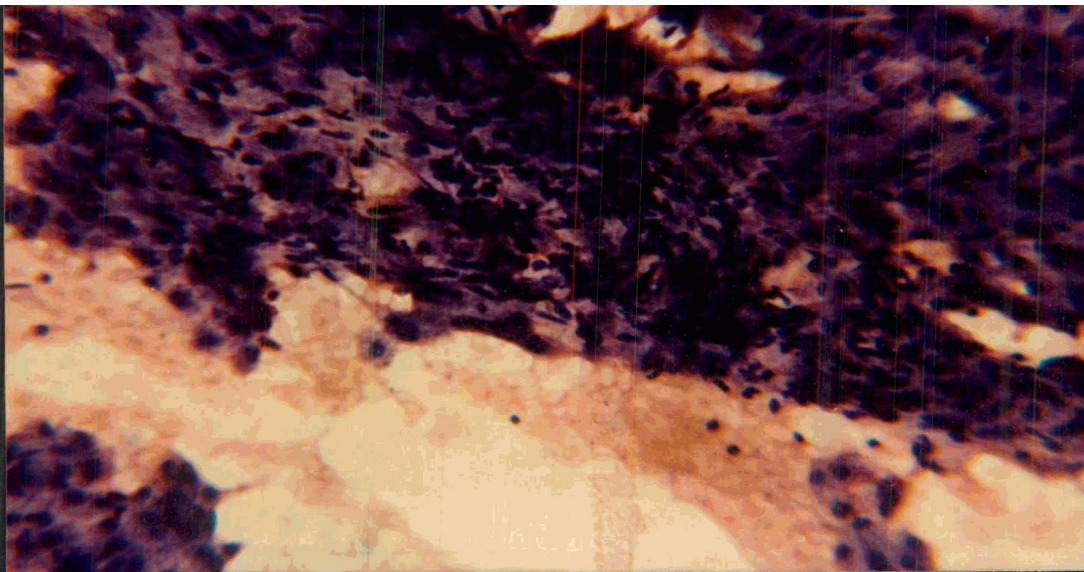
Cytologically we have diagnosed 5 cases as fatty liver aspiration and out of which 3 cases were confirmed on histologically. While one was diagnosed as fatty liver with early cirrhosis and other as CPC, on HP examination.

In fatty liver the most characteristic findings were the presence of numerous cytoplasmic vacuoles [20] and the nuclei were pushed to the periphery [photograph].

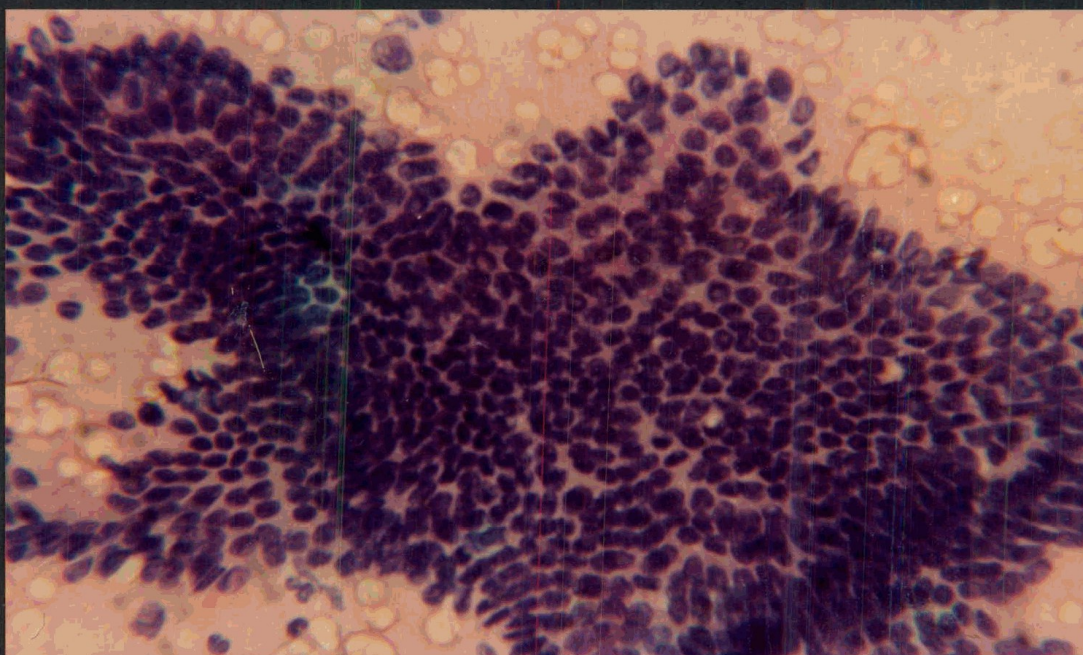
In case of CPC, liver cells show focal fatty change and necrotic hepatocytes. However, in our study we did not see necrotic hepatocytes hence diagnosed as a fatty liver. In second case which was diagnosed as fatty liver with early cirrhosis, here gross specimen did not show cirrhotic nodules. Cytomorphologically also did not reveal fibrous tissue or regenerating hepatocytes. Thus, the diagnosis of fatty liver was thought of.

3>CIRRHOTIC ASPIRATION

Cytologically 9 cases were diagnosed as cirrhosis of the liver, and all the case were finally confirmed by correlating with HP examiantion. One case showed cirrhosis with focal fatty change on HP examination, cytologically this case did not show fatty cells on smears which can be explained on the basis of focal fatty change hence the aspiration might not be representative of the lesion.



Smear showing Fibrous tissue - Cirrhosis - H & E * 192



Smear showing Bile Duct Epith. cells - Cirrhosis - H & E * 300

Our smears showed fibrocytes, many kupffer cells and also BDE cells with cellular regenerative and degenerative changes [5,20]. with single or few double nuclei, RBCs and PMN Cells seen in background with increased N/C Ratio, eccentrically located nuclei in few cases with occasional irregularity of nuclear membrane[5]. Increased fatty vacuolation were also seen [23].

4>MALARIA

One liver specimen was slate gray colored on gross, clinically it was MP positive case. Aspiration of this showed brownish granular pigments in the kupffer cells on H & E stain and also same features were found on HP examination. So, to prove that these granules were Hemozine granules and not iron pigments we had done a special stain like Prussian blue which also showed presence of brownish pigment in the kupffer cells in both cytological and histological slides, hence it was diagnosed as a malarial pigments [photographs].

5>TYPHOID

One case was diagnosed as typhoid and hence the aspiration was done which on cytological smears showed clusters of benign looking hepatocytes with bile pigments and many small clusters of large cells with round nuclei and abundant pale pink cytoplasm with distinct cell margins [s/o macrophages] with

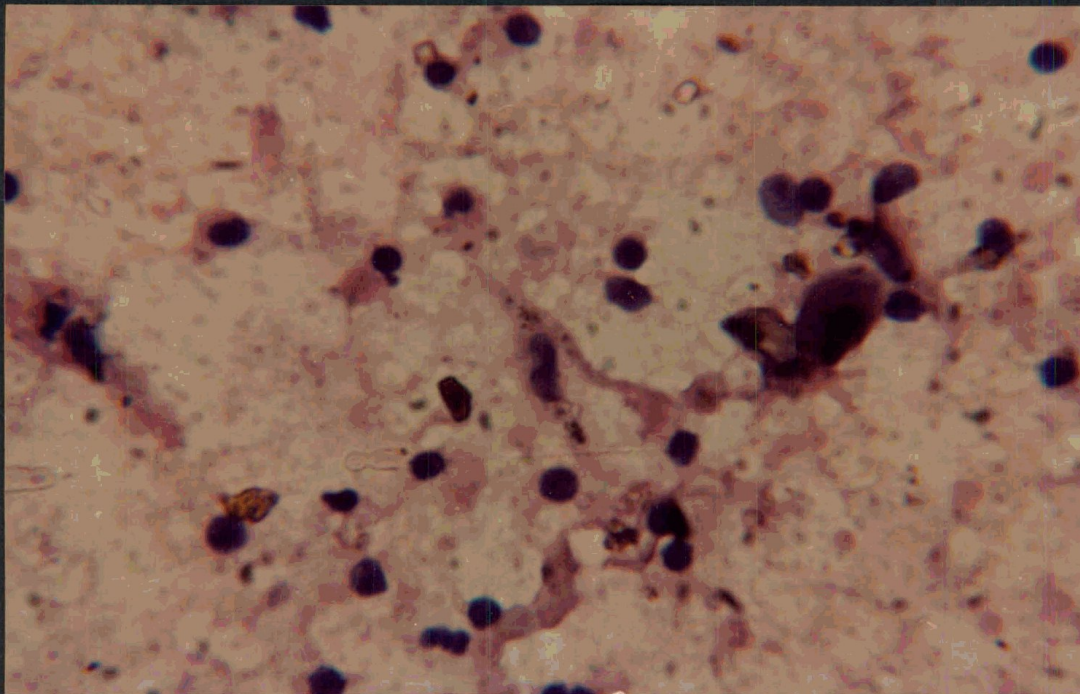
kupffer cells and fibrocytes in background . Finally the HF slide also revealed the similar features i.e. focal aggregation of macrophages and few lymphocytes, while rest was normal [photograph].Hence it was then confirmed as typhoid nodules. Histology of intestine showed typhoid ulcers.

6>NECROTIC MATERIAL [HEPATITIS]

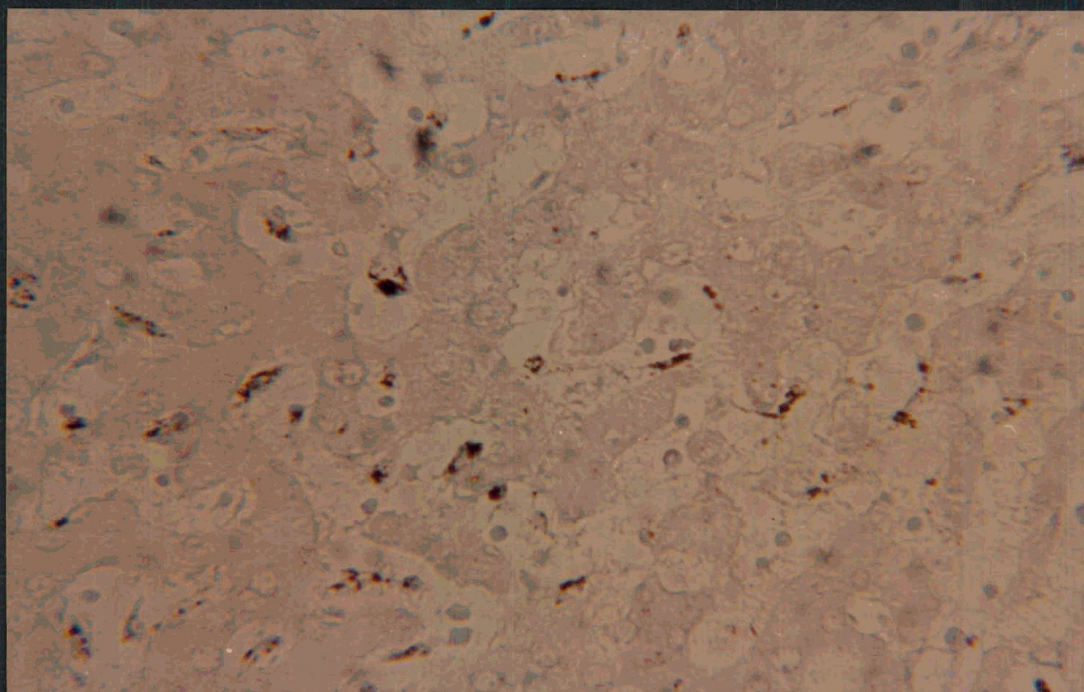
One gross specimen was showing many irregular pale areas and the aspiration from that site showed occasional cluster of benign hepatocytes while few clusters showed degenerative and regenerative changes which were in the form of double nuclei [23] and few showed necrotic changes like pyknosis,karryohexis with many MNC. Few large cells with single nuclei and moderate cytoplasm were also seen - Macrophages [23].

The HP study also showed the massive necrosis with intact peripheral hepatocytes and infiltration by MNC [23] and was diagnosed as infective hepatitis

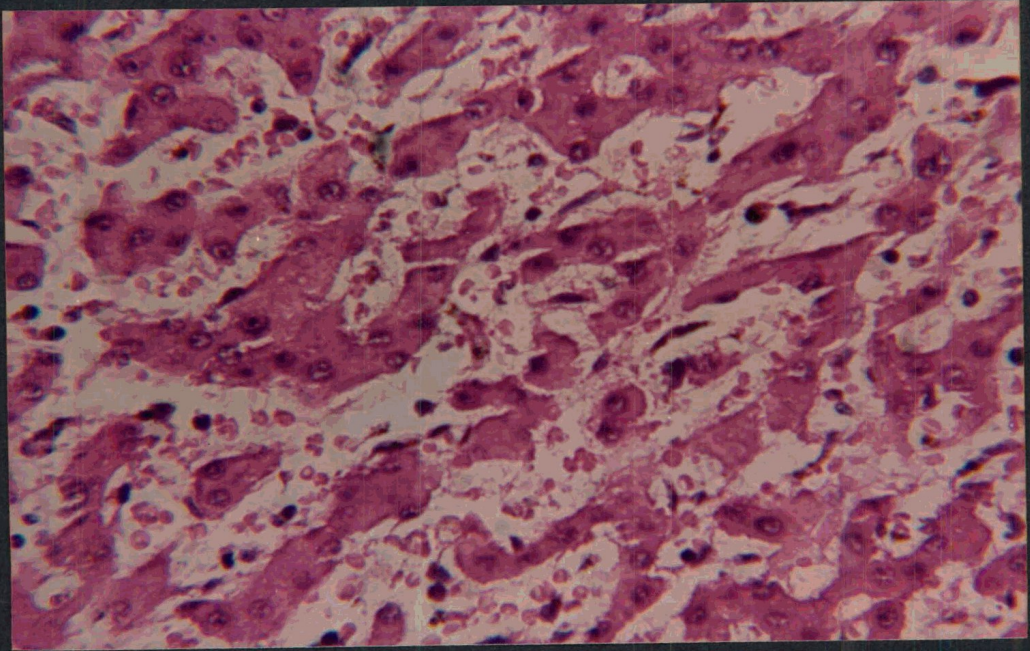
Study done by Dominis Mara, S.Cerlek and S.Dawar also showed irregular coarse and and unequally distributed chromatin and few showed chromatin concentraionn near nuclear membrane with some dark pigment in the hepatocytes [20] with lymphocytes in background they also observed macrophages in smears and most probably represented kupffer cells [20,23, photograph].



Smear shows brownish pigments in Kupffer
cells - Malaria - H & E * 300



Smear shows brownish pigments in Kupffer
cells - Malaria - Prussion Blue * 300



Section shows brownish pigments in Kupffer
cells - Malaria - H & E * 300

7>In 4 case of our aspirates the cytological diagnosis were given as fatty change with cirrhosis which finally confirmed by correlating with HP examination [8]

8>HEPATOCELLULAR CARCINOMA

In 3 aspiration the cytological diagnosis were made as HCC [1-WD, 1-MD, 1-PD] all of them finally confirmed by correlating with HP examination. One case was diagnosed as PD HCC but that was on HP diagnosed as MD HCC. One case which was diagnosed as MD HCC on FNAC turned out to be MD HCC with cirrhosis on HP examination. The cirrhotic features of this case were not appreciated on cytological smears.

Study done by Gia-Khann Nguyen [8] showed pleomorphic or large multinucleated tumor cells with scanty cytoplasm or cells with clear or vacuolated cytoplasm with bile production in minority of the cases. Globular intra or extracytoplasmic hyaline bodies were also seen [8]. In his study he also quoted that HCC is commonly associated with alcoholic micronodular cirrhosis in Europe and North America while macronodular cirrhosis of viral or toxic origin in South East Asia and Africa.

In our study we also found similar features with cells arranged in trabecular and microglandular pattern with few clusters showing elongated cells at the periphery [endothelial cells-photograph] but we have not seen the bile pigment and intra or extracytoplasmic hyaline globules in our study.

9>CHOLANGIOCARCINOMA :

A specimen on gross was showing mixed nodular cirrhosis with one large irregular mass which on cut section showed abundant scarring and purulent material. FNAC from this mass showed hepatocytes in groups as well as singly scattered with increased kupffers cells ,many lymphocytes in background, BDE cells not seen.It also showed many broad fibrous bands & thus we thought of only cirrhosis but HP study revealed it as a Cholangiocarcinoma. Here, our aspiration was negative that can be explained on the basis of extensive scarring of the lesion and hence our aspirated material showed only fibrous tissue septae and not malignant cells.

It is known that,abundant fibrous stroma is a characteristic feature of this tumor[8]. Aspirate from a cholangiocarcinoma contain variable amounts of material depending on the degree of Desmoplastic reaction in the tumor[8].

10.>METASTATIC TUMORS ;

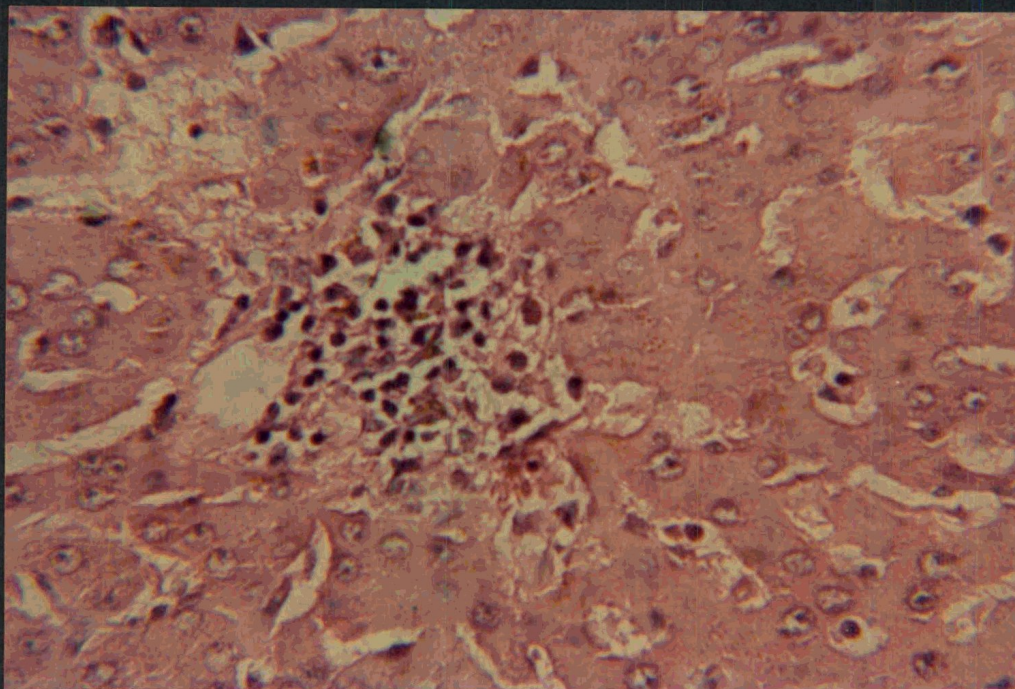
In our study of liver aspirate, 5 cases were cytologically diagnosed as metastatic tumors and all were later on confirmed by histopathological correlation. The primary were from different sites like Breast,GB, Intestine(soft tissue), Prostate,Kidney.

In case of mets from GB- the cytological smears showed cellularity with pleomorphic cells which were elongated spindle shaped & round to oval with large vesicular nuclei & prominent

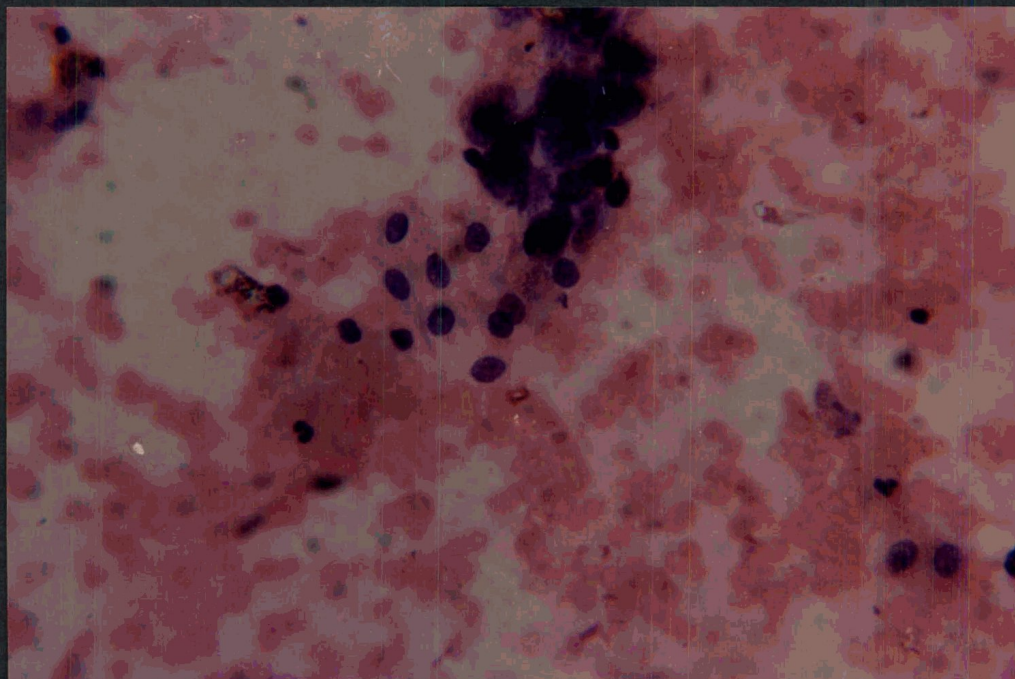
nucleoli. Cells were either in groups or singly in sheets. Few giant cells were also seen. Benign BDE cells clusters were also seen, & this was later on confirmed by HP examination as Moderately differentiated adenocarcinoma of GB

In case of Prostate carcinoma, liver mets on smears showed similar features as studied by Gia Khann Nguyun[8], many clusters of large round to oval shaped cells with large hyperchromatic nuclei, increased N/C ratio. Few normal hepatocytes with BDE cells were also seen. This was on HP examination was labelled as Adeno-squamous carcinoma, the cytological diagnosis was given as adenocarcinoma- mets from prostate. However, we have not seen the squamous elements on the smears.

One surgically resected specimen showed large nodular surface on gross which on aspiration showed cellular smears with many single elongated [scattered] cells with spindle shaped nuclei having one pointed end [photograph] and other end blunt. Few cells show coarse chromatin and tapering nuclei. No mitotic activity, hyperchromatism seen. Cytoplasm was indistinct. Few clusters of benign hepatocytes seen. The cytological diagnosis was labelled as Soft tissue tumour. On HP examination it was diagnosed as Leiomyosarcoma. Patient had a history of Leiomyosarcoma of intestine and was operated in past for the same. Female genital system were normal.



Section showing Typhoid macraphages - H & E * 300



Smear showing Typhoid macraphages - H & E * 300

Spindle cells on cytological smears suggest the diagnosis of leiomyosarcoma, fibrosarcoma, malignant schwannoma [19].

Thus it is difficult to pinpoint the diagnosis of specific type of sarcoma on cytology alone, as a pattern is not seen.

In present series - one case was metastasis of breast carcinoma and another was that from renal cell carcinoma. Cytologically as well as histopathologically both of them were correlating.

Cytologically many time it is possible to arrive at the diagnosis of the metastatic carcinoma, but difficult to suggest the primary site of the tumor. However, since it was postmortem study, all the organs were examined and we could correlate both cytology and histology.

S U M M A R Y

1> 45 cases of FNAC of liver were studied - of them 42 were postmortem cases and 3 were surgical.

2> The cytological diagnosis were given as follows....

a) Normal aspirate -- 14

b) Benign aspirate -- 22

c) Malignant aspirate -- 09

3> Histological correlation revealed

False Negative diagnosis

-- CPC 3 cases,

-- Focal fatty change 2 cases,

-- Centrilobular necrosis 1 case

-- Fatty liver with early cirrhosis 1 case

-- Hepatitis 1 case

-- HCC with cirrhosis 1 case

-- Cholangiocarcinoma 1 case

4> No False Positive cases were diagnosed.

C O N C L U S I O N

From our study it is concluded that FNAC of the liver is a safe, simple and rapid method which can be used on the wide indications. It is suitable for cytodiagnosis of malignant tumor of the liver and repeated punctures directed towards suspected changes are possible. The method is also valuable in the diagnosis of acute hepatitis, cirrhosis, typhoid and malarial involvement of the liver. A positive FNAC may prevent a laprotomy for diagnostic purposes. This will be a great benefit to the patient and saving health care resources.

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