Cytomorphological Spectrum of Hepatic Nodular Lesions
A Tertiary Care Centre Experience

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Abstracts: Background: FNAC is a very useful procedure for the diagnosis of various hepatic lesions. USG-guided FNAC offers good accuracy without major complications and minimal intervention at less cost, the only major contraindication being severe hemorrhage & vascular lesion. Although imaging techniques have helped greatly with the early and accurate diagnosis of liver abscess, the appearances are often non-specific. There is some overlap between the radiologic features of liver abscesses, HCC and metastases too. Tumors, primary or secondary, may undergo extensive necrosis, with the resultant radiologic image of the cavitatory neoplasm mimicking abscesses; abscesses are accompanied by proliferative reactive changes, making radiologic differentiation from a neoplastic process almost impossible. In these situations, FNAC plays an essential complementary role. **Material and Methods:** All patients, irrespective of age and sex, who presented to the hospital from January 2011 to June 2013 with nodular liver lesions diagnosed clinically or radiologically, with normal range of prothrombin time (International normalized ratio- INR) were subjected to fine needle aspiration under radiological guidance. Smears made were stained with toluidine blue for rapid onsite evaluation (ROSE) for adequacy of diagnostic material. Hematoxylin and Eosin (H&E), Giemsa stain and Papanicolaou stain were done as per protocol. **Results:** Of the total aspirates, 22.5% (36 cases) were benign, 63.75%(102) were malignant, 13.75% (22) non-representative as they contained only few scattered hepatocytes and blood, which was inadequate for final opinion. FNAC was of help in diagnosing liver lesions successfully in 86.25% of cases. **Conclusion:** USG guided FNAC has proved to be a fairly precise & minimally invasive technique in diagnosis of hepatic lesions as the procedure is simple, economical and easily available. The results are obtained quickly without serious complications related to the procedure. In a nutshell, FNAC is a highly accurate diagnostic tool with high sensitivity & specificity. [Selhi P NJIRM 2015; 6(1):57-61]

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Introduction: Ever since Lindquist used a fine needle aspiration biopsy (FNAB) of the liver, Johansen, Methew and other authors have presented many views on the usefulness and pitfalls of the techniques of the fine needle aspiration cytology (FNAC) of the liver for the diagnosis of both neoplastic and non-neoplastic diseases of the liver [1-3]. Zajicek stated that FNAB of the liver is a good method and perhaps better than the histological biopsy for detection of tumor metastasis and myeloid metaplasia in the liver[4]. The cellular patterns were very useful not only for diagnosis of malignancy but also for identification of the hepatic origin of the cells. The differential diagnosis of hepatic mass lesions includes primary liver tumors (benign or malignant), metastatic deposits, congenital and acquired cysts, abscesses and granulomas. [5]

The purpose of the study was to evaluate various neoplastic lesions whether primary, metastatic or non-neoplastic conditions of the liver and to correlate with histopathology whenever possible. We also tried to compare and analyze various diagnostic cytological criteria in pin pointing out a hepatocellular lesion.

Materials and Methods: All patients, irrespective of age and sex, who presented to the hospital from January 2011 to June 2013 with nodular liver lesions diagnosed clinically or radiologically, with normal range of prothrombin time (International normalized ratio- INR) were subjected to fine needle aspiration under radiological guidance. All patients were explained the procedure and Informed consent was taken prior to the procedure as per Institutional protocol for all invasive procedures.

The standard technique was applied using 22-gauge needle or long spinal needle attached to 20 ml disposable syringe. The procedure was done following all aseptic precautions during suspended respiration; needle was introduced percutaneous

into the lesion evaluated by USG. The smears made were stained with toluidine blue for rapid onsite evaluation (ROSE) for adequacy of diagnostic material. Hematoxylin and Eosin (H&E), Giemsa stain and Papanicolaou stain were done as per protocol.

Results: During the study period, 160 patients reported for aspiration of nodular liver masses. The mean age at presentation was a 55±12 year ranging from 2-86 years. The maximum numbers of cases were seen between 40-60 years of age showing a male predominance with a male to female ratio of 4:1. Radiologically, all cases had either single or multiple space occupying lesions with hepatocellular carcinoma (60 cases) being suspected in majority followed by metastasis (50 cases), abscess (10 cases) and hemangioma (4 cases).

Serum tumor markers were done in few patients with Alfa-feto protein being raised in 10 cases, CA19.9 raised in 9 cases and CEA raised in 6 cases. Clinically alcoholism, HCV and HBV were considered as the main etiological agents of the liver disease. Jaundice, followed by ascites were the leading presenting complaints. The cytomorphological features were analyzed under the following headings - cellular arrangement, cell size, N/C ratio, cell cohesion, nuclear shape and size, location, multinucleation, prominence of nucleolus, amount of cytoplasm, vacuolation, bile production and hyaline bodies.

Of the total aspirates, 22.5% (36 cases) were benign, 63.75%(102) were malignant, 13.75% (22) non-representative as they contained only few scattered hepatocytes and blood, which was inadequate for final opinion. (Table 1) .

Metastatic tumor was the most common cytomorphological diagnosis amongst the malignant liver lesions constituting 58.8% of all lesions with adenocarcinoma (NOS), squamous cell carcinoma, and melanoma being the common morphological forms. The commonest primary hepatic lesion was hepatocellular carcinoma (HCC) (41.1%). It was differentiated from benign conditions like regenerative nodule (5%), pyogenic abscess (3.75%), amebic liver abscess (1.25%), dysplastic nodule (0.625%) and reactive hepatocytes.(Table 2) The hepatocellular carcinoma was further differentiated into well differentiated (10%) and poorly differentiated (90%). (Table3)

Discussion: FNAC is a very useful procedure for the diagnosis of various hepatic lesions. In the present study cytology was of help in diagnosing liver lesions successfully in 86.25% of cases which is comparable to a study done by Franca et al. [6]

USG-guided FNAC offers good accuracy without major complications and minimal intervention at less cost, the only major contraindication being severe hemorrhage & vascular lesion. [7]

Although imaging techniques have helped greatly with the early and accurate diagnosis of liver abscess, the appearances are often non-specific. There is some overlap between the radiologic features of liver abscesses, HCC and metastases too. Tumors, primary or secondary, may undergo extensive necrosis, with the resultant radiologic image of the cavitatory neoplasm mimicking abscesses; abscesses are accompanied by proliferative reactive changes, making radiologic differentiation from a neoplastic process almost impossible. In these situations, FNAC plays an essential complementary role. Liver FNA cytology is used mainly for diagnosing hepatic malignancies, primary / metastatic. The cytological picture is not only useful for deciphering & differentiating hepatic malignancies from non-malignant entities but also guides the cytopathologist in segregating hepatic carcinomas into well/ moderately/poorly-differentiated grades. [5-7]

Three criteria differentiate HCC from metastatic tumor: Polygonal cells with centrally placed nuclei & prominent nucleoli, malignant cells separated by sinusoidal capillaries and intra cytoplasmic bile. Two additional criteria, namely, endothelial cells surrounding tumor cell clusters and intranuclear inclusions were identified as being important secondary criteria for HCC.[5, ,89] Also stripped atypical nuclei form an important differentiating feature since they are absent in non-neoplastic and metastatic lesions. Metastatic adenocarcinoma exhibits malignant columnar cells in palisaded rows

or acini, three-dimensional clusters in a background of necrotic debris sometimes with mucin secretion. [10] Neuroendocrine tumors show round nuclei with speckled granular/hyperchromatic chromatin.

The diagnosis of non-neoplastic parenchymal disease of the liver is made by identification of swollen hepatocytes, vacuolation and decreased cytoplasmic basophilia with disturbance of normal regularity of the liver cells and pronounced anisocytosis of the hepatocytes. [7]

Important cytomorphological features which help in differentiating well differentiated-HCC from a reactive liver cell can be: architectural features on the smears/cell block sections, hyper cellular smears, cohesive clusters, trabeculae, transgressing vessels/ peripheral endothelial rimming, small monotonous hepatocytes with nuclear crowding, increased nuclear cytoplasmic ratio, atypical naked nuclei & intranuclear cytoplasmic inclusions. [7-10]

In the present study, aspirates of regenerative nodules showed a polymorphous population of cells comprising hyperplastic hepatocytes, bile ductal epithelium, endothelial cells, and Kupffer cells accompanied by features of regeneration and repair. Aspirates of low-grade dysplastic nodules contain hepatocytes exhibiting large cell change with no/minimal nuclear atypia and normal nuclear-cytoplasmic ratio. Hepatocytes from high-grade-dysplastic nodules are small and monotonous with subtle increase in nuclear-cytoplasmic ratio; the nuclear size is fairly similar to that of normal hepatocytes but there is less cytoplasm, thus imparting an impression of nuclear crowding. Dysplastic hepatocytes generally occur singly or in 1- to 2-cell thick cords. Fatty change may be present. Bile ductal and ductular epithelium and stromal fragments may be evident in the background. It is difficult to distinguish high-grade dysplastic nodule and early HCC purely on cytologic grounds. [11-13]

Wee et al have given a wide range of morphological features which help in diagnosing HCC they include include (i) Hypercellular smears (ii) Irregular arborizing, broad, tongue-like cords (>2 cells thick) of cohesive malignant hepatocytes.(iii) Peripheral endothelium wrapping broad cords (iv)Transgressing endothelium running across larger aggregates : basement membrane material looking like pink “tramlines” (indicative of sinusoidal capillarization) is best seen in Giemsa preparations.(v) Cohesion is the rule: tendency to dissociation is observed in highly well-differentiated HCC due to narrow cords; and in poorly differentiated HCC where there is virtually absent reticulin.(vi) Pseudoacini containing bile or pale secretions (vii) Hepatocytic characteristics include polygonal cells with well-defined cell borders, ample dense granular cytoplasm, increased nuclear-cytoplasmic ratio (>1/3), central round nucleus, well-delineated nuclear membrane, prominent nucleolus, and fine, irregularly granular chromatin.Mitoses increase with nuclear grade: cytologic features of malignancy are wanting at the well-differentiated HCC end whereas clues to hepatocytic histogenesis are lacking at the poorly differentiated end.(viii) Tumor cells may be smaller, larger, or of the same size as nonneoplastic hepatocytes : well-differentiated HCC cells tend to be conspicuous by their small size, monotony, subtle increase in nuclear-cytoplasmic ratio and nuclear crowding. Poorly differentiated HCC cells tend to be pleomorphic with thin nuclear membranes and irregular nuclear contours.(ix) Atypical bare hepatocytic nuclei may abound .(x) Multinucleated tumor giant cells may be of “osteoclastic” or pleomorphic type : the former shows nuclear features akin to sibling tumor cells. Tumor giant cells may be found even in the lower grades of HCC. Their presence does not necessarily upgrade the tumor.(xi) Bile may be present within tumor cells or in canaliculi or pseudoacini : bile appears as greenish-black intracytoplasmic droplets, ropey intracanalicular strands and blobs within pseudoacini; best detected in Giemsa-stained smears.(xii) Intracytoplasmic fat and glycogen vacuoles are common. Intracytoplasmic inclusions include hyaline, pale, and Mallory bodies . Intranuclear cytoplasmic inclusions are not specific.(xiii) Bile duct epithelial cells, if present, are few and far apart. Background may be hemorrhagic and/or necrotic.[12]

In the present study we tried to grade the hepatocellular carcinomas into well, moderate and poorly differentiated lesions based on nuclear grade. Lin C et al and others have supported this cytologic grading with well-differentiated HCC tumor cells closely resembling nonneoplastic hepatocytes in size, shape and nuclear and nucleolar appearances. The nuclear-cytoplasmic ratio is slightly higher. Mitoses are exceptional. While moderately differentiated HCC tumor cells bear a resemblance to nonneoplastic hepatocytes. The nuclear-cytoplasmic ratio is moderately high, the round to ovoid nuclei show moderate degrees of pleomorphism, nucleoli are prominent, and mitoses are identifiable and poorly differentiated HCC tumor cells exhibit marked pleomorphism, less cytoplasm, very high nuclear-cytoplasmic ratios, thinner nuclear membranes with irregular nuclear contours, hyperchromasia, and numerous mitoses. Nucleoli may be prominent or absent. Multinucleated tumor giant cells are easily identified.[13,14]

With advances in cytology techniques like cell block are being promoted where sections simulate histologic sections and routine stains and immunohistochemical stains can be useful in diagnosing lesions using FNAC ; a less invasive method as compared to biopsies. [15]

An armamentarium of antibodies is available for the comparative immunohistochemical analysis of primary and metastatic tumors of the liver. Careful light microscopic assessment of the histologic/ cell block sections is important as judicious use of immunostains is imperative since material is limited. Kakar et al. Outlined best practice guidelines for use of immunohistochemistry in the differential diagnosis of hepatic lesions under specific clinical scenarios . Stepwise logistic regression analysis has shown that the panel of glypican-3, HepPar1, MOC-31, and CK7 is most useful in diagnosing and distinguishing HCC from metastatic adenocarcinoma on FNA material, with accuracy rates of 90.5 and 91.7%, respectively . In the HCC group, glypican-3 was the most sensitive (81%), whereas HepPar1 (71.4%) and polyclonal carcinoembryonic antigen (pCEA) (50%) were less sensitive. In the metastatic adenocarcinoma group, MOC-31 was most sensitive (79.2) followed by CK7 (41.7%).

In the context of hepatocellular nodular lesions, the objectives are twofold: (i) to prove hepatocellular histogenesis and (ii) to demonstrate the malignant status of the hepatocytes. For the former, the panel should include Hep Par 1 , TTF-1 , and pCEA or CD10 to demonstrate canalicular formation. For the latter, the panel should include glypican-3, glutamine synthetase (a target protein of β-catenin), and heat shock protein 70 (a chaperone stress protein); two out of three positivity of these novel biomarkers are taken as indicative of HCC.[16-19]

Conclusion: The role of fine needle aspiration cytology fits into the overall patient clinical pathway as FNA biopsy offers the potential immediacy of a diagnosis to the clinician who can then advise the patient and develop an appropriate next clinical step. The rapid turn-around time or at best reporting within 24 hours is ideal but for most practices this is not achievable with current resources. On-site cytology service provides immediate evaluation for adequacy and triage of specimens, which can be assessed by cytotechnologists rather than cytopathologists. The reduction in inadequate sample rates is important for overall cost effectiveness of the technique.

The FNA biopsy technique is still the most minimally invasive approach for the procurement of tumor and peritumoral tissue for molecular studies. We foresee that in the near future hepatic FNA is likely to become a point of care in the management of HCC patients, especially inoperable cases. In the present setup from this study it is felt that USG guided FNAC is a very useful in diagnosis of different hepatic lesions as the procedure is simple and safe. The results are obtained quickly without serious complications related to the procedure. So, FNAC is a simple and effective diagnostic tool in our hand.

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