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ORIGINAL ARTICLE

Cytological Differential Diagnosis Criteria of Liver Masses

Davut Şahin

Department of Pathology, Acıbadem Health Groub, Istanbul, Turkey

Abstract

Introduction: The objective of the study was to determine the critical diagnostic cytological features of the liver masses.

Methods: This retrospective study was conducted with 137 fine needle aspiration biopsies diagnosed in a 5-year period in Haydarpaşa Numune Hospital. All the glass slides of the cases were re-evaluated and 11 cytomorphologic features were investigated in each case. The results were evaluated by Stepwise Logistic Regression Analysis method.

Results: Monotonous atypia, atypical hepatocytic naked nuclei, increased nucleus/cytoplasm (N/C) ratio, and hepatocytic morphology were critical for differential diagnosis of hepatocellular carcinoma (HCC) and metastasis. Increase N/C ratio and the absence of bile duct epithelial cells were critically important for differential diagnosis of HCC from benign liver mass.

Discussion and Conclusion: Cytological diagnosis of liver mass is not difficult in cases where cytomorphological features are sufficient. The efficacy of cytology in the differentiation of benign liver masses from HCC is limited. This can be achieved by performing immunohistochemistry to the cell block. Cytopathologic diagnosis should be confirmed with radiological and biochemical results.

Keywords: Cytologic differential diagnosis; fine-needle aspiration; liver masses.

Fine-needle aspiration biopsy (FNAB) is one of the methods used in the diagnosis of liver masses[1,2]. The method has advantages such as easy application, evaluation in a short time and being repeatable when necessary and absence of serious complications. There are two main problems in liver cytology. One of them is the differential diagnosis of benign lesions and hepatocellular carcinoma (HCC), the other is the differential diagnosis of HCC and metastatic carcinomas[3-5]. Cytomorphological features of benign liver lesions such as cirrhosis, regenerative hyperplasia, focal nodular hyperplasia, and hepatocellular adenoma are very similar to well-differentiated HCC[6-8]. Because of these similarities, difficulties are encountered in the differential diagnosis of benign lesions and well-differentiated HCC. In poorly differentiated HCC, the cells do not have hepatocytic morphology and their malignant characteristics can be easily recognized, but the differential diagnosis of the lesion from metastatic carcinomas is difficult[5]. Another problem is the differential diagnosis of cholangiocarcinoma and metastatic adenocarcinoma. Quite different results have been reported in studies conducted to determine cytological features that are important in the differential diagnosis of benign lesion/HCC and HCC/metastasis[9,10].

The aim of this study is to determine the rate of cytological features used in the diagnosis of liver masses, such as benign lesions, HCC, and metastatic carcinomas, and to determine the most important of these features in cytological diagnosis.

Correspondence (İletişim): Davut Şahin, M.D. Acibadem Saglik Grubu Patoloji Laboratuvari, Istanbul, Turkey
Phone (Telefon): +90 532 247 43 82 **E-mail (E-posta):** davutpato@gmail.com
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Materials and Methods

In this retrospective study, 137 livers FNAB performed over a 5-year period at Haydarpaşa Numune Hospital were examined. During this period, the number of cases with liver FNAB was 197. Sixty cases with diagnoses such as hydatid cyst, abscess content, and non-diagnostic result were excluded from the study.

All aspirations were performed with the assistance of radiological imaging such as ultrasonography or computed tomography. 20–23 gauge needles and 10 cc plastic sterile injectors were used for aspirations. Conventional smear preparations were prepared from the aspirations, some of them were fixed in 95% ethyl alcohol and stained with Papanikolaou; others were dried in air at room temperature and stained with May-Grunwald-Giemsa. Cell blocks were cut at 5-micron thickness and stained with Hematoxylin and Eosin. During the FNAB procedure, one of the smear preparations was stained with Toluidine blue dye and was evaluated by a pathologist under the light microscope.

The distribution of cytological diagnoses according to the groups was as follows: 25 HCC, 55 metastatic adenocarcinoma and cholangiocarcinoma, 23 other metastatic malignancies, four cirrhosis, 16 hepatocytes with reactive-regenerative changes secondary to inflammation, one dysplastic nodule, one biliary ductal epithelial cell, one regenerative nodule, and 15 normal hepatocytes.

A pathologist re-evaluated the preparations of previously diagnosed and cytologically diagnosed cases under the microscope to determine the incidence of the investigated cytological features. Eleven cytological features used in the differential diagnosis of benign/malignant, HCC/metastatic liver masses were investigated in the preparations of all cases. The features investigated were coded as positive (+) if present, as negative (–) if not, and the data were entered into a SPSS data table. The results were evaluated using the "Stepwise Logistic Regression Analysis" method.

The cytological features investigated were as follows: (1) Monotonous atypia (MA), (2) Trabecular pattern, (3) Acinar pattern, (4) Nucleus/Cytoplasm (N/C) ratio increase, (5) Atypical hepatocytic naked nuclei (AHNN), (6) Hepatocytic appearance (HA), (7) Bile, (8) Benign hepatocytes, (9) Bile ductal epithelial cells (BDEC), (10) Nuclear overlapping, and (11) Intranuclear inclusion (INI).

Results

The distribution of the cases according to the diagnosis groups, the mean ages and genders of the patients are shown in Table 1.

The rates of the 11 investigated cytomorphological features in benign and malignant liver lesions are shown in Table 2.

In the differential diagnosis of HCC and metastatic carcinomas, the increase in MA, AHNN, N/C ratio, and HA was found to be of decisive importance.

Discussion

FNAB is a diagnostic method used in the diagnosis of liver masses, with a sensitivity of 55–100% and a specificity of 87.5–100%[11-13]. In recent years, an increasing number of

Table 1. Distribution of the cases by diagnosis groups, age, and gender characteristics

Cytological diagnosis	Number	Mean age	Female/Male
Hepatocellular carcinoma	25	60.7	6/19
Adenocarcinoma metastasis	51	57.6	25/26
Small cell carcinoma metastasis	3	64.0	0/3
Epidermoid carcinoma metastasis	3	55.0	1/2
Malign melanoma metastasis	3	64.5	1/2
Malignant lymphoma	2	67.0	0/2
Poorly differentiated carcinoma me	10	52.0	5/5
Undifferentiated malignant tumor	1	65.0	1/0
Carcinoid tumor	1	41.0	0/1
Cirrhosis	4	66.2	1/3
Reactive-regenerative hepatocytes	16	57.3	7/9
Atypical hepatocytes	1	55.0	0/1
Biliary ductal epithelium	1	55.0	0/1
Nodular regenerative hyperplasia	5	73.0	1/4
Benign hepatocytes	15	55.3	5/10

Table 2. The rates of the investigated cytological features in HCC and benign liver lesions

Cytological feature	HCC		Benign lesions	
	Number	Incidence rate (%)	Number	Incidence rate (%)
Monotonous atypia	15	60	0	0
Hepatocytic appearance	21	82	38	100
N/S ratio increase	25	100	1	2
Atypical hepatocytic naked nuclei	21	82	27	71
Bile	11	42	30	73
Intranuclear Inclusion	14	56	17	45
Trabecular pattern	18	61	5	12
Acinar pattern	2	8	2	5.2
Benign hepatocytes	4	16	38	100
Ductal epithelium	1	4	14	36
Overlapping	25	100	1	2.5

HCC: Hepatocellular carcinoma.

studies on liver fine-needle aspiration cytology performed under the guidance of endoscopic ultrasonography have been reported[14-17]. In liver cytology, difficulties are encountered in the differential diagnosis of benign lesions such as cirrhotic nodules, regenerative nodular hyperplasia, focal nodular hyperplasia, hepatocellular adenoma, and well-differentiated HCC, metastatic carcinomas, and poorly differentiated HCC[3-5,10,18]. In cases, where tumor differentiation is poor and the origin of the cells cannot be determined by cytomorphological findings, immunohistochemical and biochemical examinations are required[19-22]. Cytological differential diagnosis of cholangiocarcinoma and metastatic adenocarcinomas is also impossible in most cases, and this distinction is made with clinical, radiological, and laboratory results.

MA, which is reported to be between 47% and 60% in HCC in the literature, was present in 60% of our cases[11,12]. MA is characteristic of particularly well-differentiated HCC and is seen at lower rates in poorly and moderately differentiated HCC (Figs. 1, 2). Apart from HCC, MA can also be seen in small cell carcinoma, carcinoid tumor, round cell malignant tumors, and malignant melanoma[11].

In the literature, the presence of trabecular structures with a width of four or more cells is reported to be critical for the diagnosis of well-differentiated HCC, and it has been reported to be seen at a rate of 65–77% in HCC and 10% in benign liver lesions[10-12]. According to our results, trabecular pattern was present in 61% in HCC. Except for one undifferentiated malignant tumor metastasis, this feature was not seen in other metastatic tumors. In addition, trabecular structures with a width of 2–3 cells were observed in three

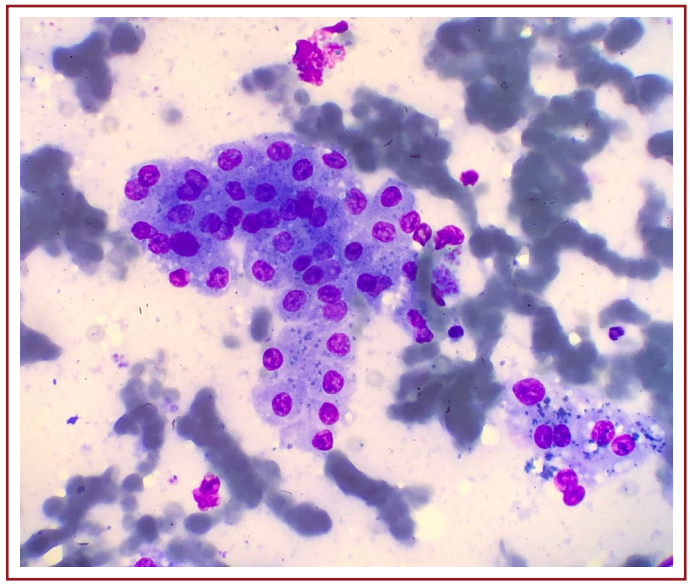

Figure 1. Normal liver parenchymal cells (May-Grunwald-Giemsa ×400).

benign liver lesions, two diagnosed with cirrhosis, and one with nodular regenerative hyperplasia (NRH). It is reported that trabecular structures occurring in benign liver masses such as hepatocellular adenoma, focal nodular hyperplasia, and NRH are 2–3 cells wide[5]. NRH, which shows atypical changes, is one of the leading benign lesions that cause difficulty in differential diagnosis with HCC. Cytological abnormalities are more noticeable in HCC, and nuclear atypia, nucleolar, trabecular, and acinar pattern are more common and prominent. The width of trabeculae and nuclear properties is not sufficient for the differential diagnosis of NRH and HCC. Trabeculae are 1–2 cells wide and nuclear atypia may be minimal in early stage HCC. In necessary cases, im-

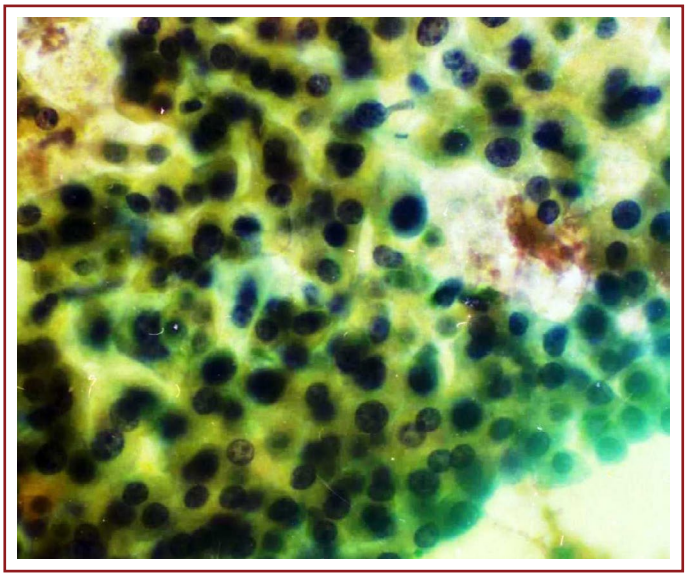

Figure 2. Malignant liver cells with chaotic sequence and high nucleus/cytoplasm ratio. Hepatocellular carcinoma (PAP ×400).

munohistochemistry and molecular analysis can be done for differential diagnosis[19-22]. Thick needle biopsy may be required for definitive diagnosis of problematic cases. It is reported that the most specific diagnostic tool in benign lesions is thick needle biopsy[7].

It is reported in the literature that there are 31–38% acinar structures in HCC[12,13]. In our results, this rate was 8%. The reason for the difference between our results and literature data may be the small number of our cases, insufficient sampling, or the difference in interpretation in microscopic evaluation. Due to the presence of acinar structures in HCC, metastatic adenocarcinomas and cholangiocarcinomas, cytological differential diagnosis among these lesions is not easy. Searching for signs specific to HCC, such as the HA of the cells, the presence of bile, and trabecular pattern, aids in the differential diagnosis. According to our results, 37% of adenocarcinoma metastasis cases had acinar structure.

In the literature, the incidence of AHNN in HCC is reported as 15–90%[11-13]. This rate was 84% in our cases. About 32% of our cases with benign liver lesions had AHNN. It is reported that this feature is an artifactual change caused by late fixation[23].

HA, a feature of well-differentiated HCC, is less common in moderately and poorly differentiated HCC[12,13]. This feature, which was reported to be 66–80% in HCC in the literature, was present at a rate of 84% in our cases. Cytological differential diagnosis of poorly differentiated HCC, which has lost its HA, from metastatic carcinomas may be impossible. In such cases, immunohistochemical markers showing hepatocytic differentiation should be applied to the cell block[22].

There was an increase in N/C ratio in all our HCC cases (100%). In the literature, this feature is reported to be 75–100% in HCC[11-13]. N/C ratio increase was observed in all of our metastasis cases except one malignant melanoma and one metastatic adenocarcinoma case. Except for one case with severe atypical changes, N/C increase was not observed in our benign liver lesions. With these data, it can be said that the increase in N/C ratio in FNAB cytology of liver masses is an important feature indicating that the lesion is malignant.

In the literature, it is reported that intracellular or extracellular bile is seen in 43% of HCC[10-12]. Our results showed the presence of bile in HCC at the same rate (43%) as in the literature. For the differential diagnosis of HCC and metastatic carcinoma, it can be said that bile pigment is the most specific cytological feature, although its sensitivity is not very high. This is because there is no other malignant tumor containing bile other than HCC. Since there is bile in the cells of benign liver masses, this feature has no value in the differential diagnosis of benign liver lesions and HCC[3-5]. There may be intracytoplasmic melanin pigment in malignant melanoma metastasis. In microscopic evaluation, melanin and bile differentiation cannot be made 100%. Therefore, immunohistochemical markers or histochemical dyes showing melanin can be used for the differential diagnosis of HCC and melanoma.

INI is reported to be found in HCC at a rate of 18–71%[7,11,23]. Our results are compatible with the literature and there was 44% INI in HCC. Two melanoma, one undifferentiated carcinoma and five adenocarcinoma metastases cases also had INI. Malignant melanoma may mimic HCC with features such as intracytoplasmic melanin pigment, INI, and epithelioid morphology and cause misdiagnosis. However, INI is one of the clues that the tumor is of hepatocyte origin in poorly differentiated HCC[23].

In our four HCC cases (16%), there were normal hepatocytes along with malignant tumor cells. In the aspiration of small-sized masses, reasons such as sampling of surrounding normal tissue or sampling of benign hepatocytes in the line where the needle passes may be the reason for this result[23].

In the literature, it is reported that BDEC is not seen in HCC[9-11]. Our results showed that BDEC was 4% in HCC and 36% in benign liver lesions. The reason for having 4% BDEC in HCC may be sampling of surrounding non-neoplastic tissue during aspiration.

Cohen et al.[8,13] reported that the three cytological cri-

teria that are key in the differential diagnosis of non-neoplastic liver lesions and HCC were N/C ratio increase, trabecular pattern, and AHNN. Our results differed from those of Cohen et al.[8,13] and showed an increase in N/C and the absence of BDEC as the determining cytological features in the differential diagnosis of benign liver lesions and HCC. It has been reported that benign liver masses such as hepatocellular adenoma, focal nodular hyperplasia, and macroregenerative nodules also have a trabecular pattern[5-7]. AHNN is not a specific feature, and there may be hepatocyte-like naked nuclei in many lesions such as benign and malignant liver lesions, melanoma, and metastatic carcinoma. In the study of Cohen et al.[8,13] and in our study, it was seen that the important common finding for the cytological differential diagnosis of benign hepatic lesions and HCC was the increased N/C ratio.

In a study conducted to determine the key cytological features in the cytological differential diagnosis of metastatic carcinoma with HCC, bile pigment, trabecular pattern, and HA were reported as the three most important features. [5] In one of the studies conducted for the same purpose, irregular sequence (loss of polarity), abnormal chromatin and MA in hepatocytes, and in another study, bile, trabecular pattern, and intracytoplasmic Mallory body trio were reported as the most important features[8,10]. According to our results, the most important cytological features in favor of HCC in the differential diagnosis of HCC and metastasis were MA, N/C increase, HA, and AHNN (Table 2). The most important features we found for the differential diagnosis of benign hepatic lesions with HCC were the increase in N/C and the absence of bile duct epithelial cells. N/C increase in the differential diagnosis of benign hepatic lesions and HCC, and HA in the differential diagnosis of HCC and metastatic carcinomas were the key common cytological features between the literature and our results.

Conclusion

Although the application of FNAB in the diagnosis of liver masses has decreased in recent years, it still continues due to reasons such as the widespread use of endoscopic ultrasonography, the localization of the mass not suitable for thick needle biopsy, and staging of malignant tumors[15-17,24,25]. While the differential diagnosis of benign hepatic lesions and HCC, HCC and metastatic carcinoma is easy in cases with all the determining cytomorphological features in liver cytology, it is difficult in cases where these features are partially found. Our results showed that four common cytological features that were common in HCC were MA, AHNN, HA and N/S increase. In benign liver lesions, BDEC and hepatocytes with normal appearance were found to be the determining diagnostic cytological features. In cases, where cytomorphological criteria are not sufficient, immunohistochemistry can be applied to the cell block for definitive diagnosis or confirmation of the cytological diagnosis[20-22]. Thick needle biopsy can be performed in cases where cytological diagnosis cannot be made. Cytopathological diagnosis should be confirmed by radiology and biochemical results.

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Conflict of Interest: None declared.

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