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Diagnostic Value of Ki-67LI in Hepatocellular Carcinoma

Ki-67 Antigen Expression in Hepatocellular Carcinoma, Metastatic Adenocarcinoma and Normal Hepatocytes

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Abstract: One hundred liver fine-needle aspiration cytology (FNAC) specimens obtained from 42 patients with hepatocellular carcinomas (HCC), 38 patients with metastatic adenocarcinomas (MAC) and 20 patients with non-malignant liver disease (control group) were studied. Liver specimens were stained with an immunoperoxidase method using a monoclonal antibody to Ki-67 (MIB1).

Ki-67 antigen was visualised in cold acetone, and a fresh liver needle aspiration cytology specimen using antibodies was used to identify proliferating hepatocytes. In this study it was examined that; if there is a difference between the groups in terms of Ki-67 positivity and degree of differentiation of tumor and the relationship there in.

The Ki-67 labeling index (LI) of HCC cases was significantly different from the other two groups ($p < 0.01$). In additions, the numbers of hepatocytes positive for Ki-67 has a good correlation with the degree of differentiation of HCC ($t = 2.96$, $p < 0.01$).

Key Words: Hepatocellular carcinoma, Ki-67 antigen, fine-needle aspiration, cytology

Introduction

Established prognostic factors in hepatocellular carcinomas (HCC) are: stage, tumor size, capsule formation, capsule invasion, number of tumor nodules, histologic subtype, mitotic index, differentiation, presence of cirrhosis in patient, AFP level in serum, level of plazma des-γ-carboxy prothrombin, tumor thrombus in the portal vein, intrahepatic metastasis, and the proliferation potential of tumor (1-8). In order to find out the proliferation potential of tumor, proliferating cell nuclear antigen (PCNA) (4-7) and p53 (9) were frequently used with overexpression histologic slides and cytologic materials, but because of some difficulties in applying it to tissue, Ki-67 was used less frequently (10-14).

The current study is composed of two parts and has two aims. One is to establish whether the Ki-67 labeling index (LI) of liver fine-needle aspiration cytology (FNAC) is a discriminating factor between HCC (42 cases), metastatic adenocarcinoma (MAC) (38 cases) and the control group (20 cases) or not. The other aim is to establish the relationship between Ki-67 LI in HCC cases and prognostic factors.

Materials and Methods

FNAC was performed by a clinician using Computed Tomography (CT) or ultrasound guidance with a 22-gauge spinal needle. The aspirated material was immediately smeared onto between 4-20 glass slides. The cellular slides are selected by direct visual inspection for routinely examination and 2 of these are chosen for immunocytologic examination. These slides are fixed in cold acetone. The air-dried smears were stained with May-Grunwald-Giemsa (MGG) stain.

Fine-needle aspiration from 42 HCC, 38 MAC cases and 20 benign lesions were re-examined. The diagnosis was confirmed by tissue examination (28 patients; 66.6%), or by clinical and laboratory findings alone in HCC patients. All of the patients who had metastatic liver lesions were eventually proven to have hepatic malignancy following at subsequent tissue biopsy. There was no malignancy in the reactive group.

The cellular structure of HCC cases was categorised under three headings. These were well-differentiated cell type (WDCT), pleomorphic large cell type (PLCT), and poorly differentiated cell type (PDCT).

For the immunocytochemical stain, Ki-67 antigen was applied to 100 liver FNAC, which included 42 HCC cases, 38 MAC cases and 20 non-neoplastic control cases.

The samples were incubated in 0.3% hydrogen peroxidase for 15 min. to block endogenous peroxidase activity, and incubated with Ki-67 monoclonal antibody MIB1 (DAKO 1:50 dilution) for 60 min. Ki-67

immunostaining was performed using a LSAB kit (DAKO) and was demonstrated with a second-stage biotin-conjugated antibody, with slides incubated for 30 min., followed by peroxidase-conjugated streptavidin for 30 min. All steps were performed at room temperature and followed by washing in phosphate-buffered saline. Peroxidase activity was detected with 3.3-diaminobenzidine tetrahydrochloride in Tris-buffered saline, pH 7.6 for 5 min. The slides were counterstained with hemotoxylin.

The MIB1 labeling index was calculated as the number of hepatocytes counted by two observers, counting at least 1.000 cells. All the stained cells were considered positive regardless staining type (homogenous, along the nuclear membrane,etc.) and degree of staining.

Statistics

In order to see if there was a significant difference between the HCC, MAC and control groups, one-way ANOVA and post-ANOVA techniques were used. The degree of differentiation in HCC tumors and Ki-67LI were examined by Student's t-test.

Results

The mean of age 52.80 in HCC cases. The youngest patient was 34, and the oldest 76; 90 % of the cases were male. There was no relationship between sex and Ki-67LI ($P > 0.05$). Ki-67LI was (mean ± standard deviation) 53.75 ± 35.44 for female patients and 39.60 ± 31.00 for male patients. Hepatitis B surface antigen (HBsAg) was positive in 65% of HCC cases. Eight of the cases were cirrhosis. There was chronic alcohol consumption in 6 of the cases. The AFP level in serum was high in all HHC cases. The smallest tumor in the cases was 30 mm in diameter. In 11 cases the tumors were located on the right lobe, on the left in 8 cases and diffuses in 23. In 4 of the cases, there were local recurrences within a year of surgery.

In HCC cases Ki-67 LI was 40.95 ± 31.25, in MAC cases 24.86 ± 28.17, and in the control group 17.00 ± 21.66. Different types of staining were observed such as diffuse, granular, along the perinuclear membrane etc. The amount of staining among the cases was observed to have changed. In some cases mostly cytoplasmic staining was observed including reactive cells. Ki-67LI in HCC cases was found significantly different than in the MAC and reactive groups ($p < 0.01$). Among the 38 metastatic adenocarcinoma cases stained by Ki-67, there were 3 cases metastatic colon adenocarcinoma, 2 of metastatic stomach adenocarcinoma cases, 2 of mucinous adenocarcinoma originating from the gastrointestinal system, 2 of metastatic pancreas adenocarcinoma, 2 metastatic breast carcinomas, and 2 cases of metastatic adenocarcinoma originating from the lung. The primary cause of 25 cases could not be found.

Of the 42 HCC cases, stained by Ki-67, 29 were WDCT, 12 PDCT and 1 PLCT. Of these cases, the only special-type PLCT HCC case was kept out of the statistical analysis. WDCT cases were compared to PDCT in terms of Ki-67LI. Ki-67LI was 32.06 ± 29.01 in the first group (n=29) and in the second (n=12) 60.83 ± 28.74. The results show a significant difference between the groups ($t = 2.96$ $p < 0.01$)

Ki-67LI in HCC cases and some variables such as the age of the patient, sex, location of tumor, diameter of tumor, tumor invasiveness, AFP level, presence of HBsAg, presence of cirrhosis and alcohol consumption were analysed, but no significant relationship between the variables could be found ($p > 0.05$).

Discussion

There have been some difficulties in differentiating HCC from reactive cells and metastatic tumors by FNAC. There are some studies in the literature showing the importance of proliferation indicators in differentiating HCC from reactive cells and MAC (15). In order to do that, proliferating cell nuclear antigen (PCNA) and P53 are usually used because of their ease of application in tissue. Among these indicators, Ki-67 is a monoclonal antibody, which was developed by Gerdes et al in 1983 (16,17). Although Ki-67 has some application difficulties in tissue, liver specimens taken by FNAC are treated as frozen and the application is carried at easily. Since FNAC specimens are dried in the air, they are accepted as fresh tissue, and do not therefore need to be treated with tripsin in the microwave before applying the antibody. In this way, money, time and effort can be saved. The other techniques used to find out the growth ratio of tumors, such as like tritiated thymidine, bromodeoxyuridine incorporation and flow cytometry are difficult and expensive. Ki-67 was applied in 100 cases and we aimed to see if Ki-67LI can be used as an indicator of HCC and wheather it is a differentiating feature of reactive cells

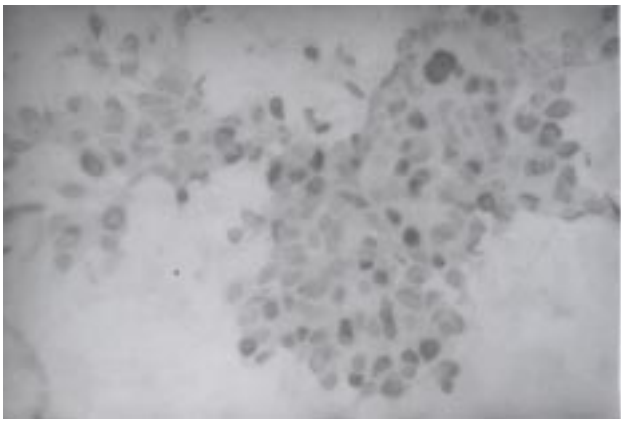

Figure 1. Ki-67 staining in HCC (Immunperoxidase x200).

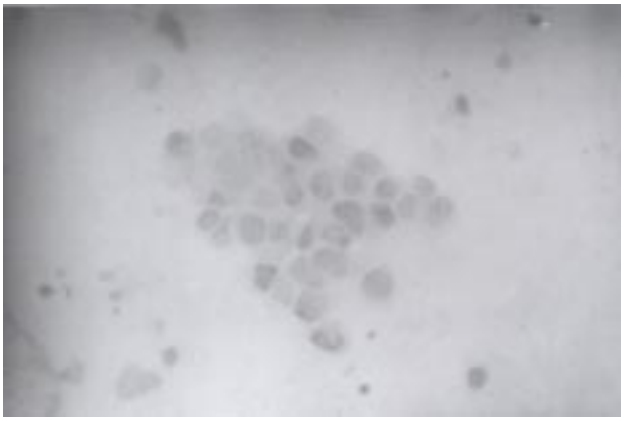

Figure 2. Ki-67 staining in HCC (Immunperoxidase x200).

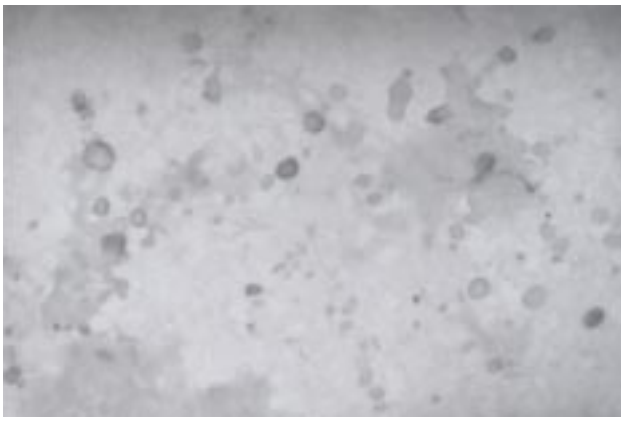

Figure 3. Ki-67 staining in MAC (Immunperoxidase x200).

and metastatic tumors. Another purpose of the study is to determine the relationship between by Ki-67LI and other prognostic factors of HCC cases.

There were three groups in this study. The first consisted of 42 HCC cases, the second was the MAC group with 38 cases and the last was the control group that comprised 20 liver specimens with no atypical cells. Ki-67 was applied to all the groups. When the Ki-67LI results were compared statistically significant difference emerge between the HCC and the other two groups ($p < 0.01$).

Proliferating lesions in liver have been known in a high spectrum (4). At one end of this spectrum, there is a regenerative nodule, and at the other end there is poorly differentiated HCC. In our study, 3 cases were diagnosed as regenerative nodules in histopathologic examinations. The level of labeling nucleus by Ki-67 in these cases was lower than 25%.

In diagnosing HCC, there might be some confusion between poorly differentiated HCC and MAC. In our study there were 12 HCC cases in PDCT. In nine of these cases, the level of Ki-67 staining was higher than 50%. In seven of 38 MAC cases, the staining level was higher than 50%. In histopathologic examination, one case that was diagnosed as HCC had not been categorised as PDCT HCC and MAC during cytologic examination. This case received 80.2% labeling nucleus by Ki-67 and was quiet prominent.

Many factors determine prognosis in HCC. Most of the cases stained by Ki-67 were in the 5th and 6th decades. The male/female ratio was 38/4. No significant relationship between sex, age and Ki-67LI was found ($p > 0.05$). In the literature, it is known that the prognosis is better and the ratio of reoccurrence lower in women than men (4,10). The reason: this is thought to be the higher probability of tumor in the capsule and lower tumor invasiveness in women (4). In addition some clinical (18,19) and experimental studies (20) have shown that if a patient is treated with androgens and estrogens HCC may occur in that patient. However the effect of tamoxifen and other estrogen receptor blockers on the survival of hepatocellular carcinoma is contraversial (21,22). In our study, in only one male case was there a tumor in the capsule. We had no cases that had been treated with hormones or used hormone receptor blockers.

In HCC cases, the other two important prognostic factors are the level of alpha-fetoprotein in serum (AFP) and level of plasma des-γ-carboxy (8). In this study, AFP levels were higher than usual in all cases. There was no significant relationship between Ki-67LI and AFP levels ($p > 0.05$).

In HCC cases, although the tumors were of an operable size it is known that prognosis was poor in the postoperative period (23). The most important cause of death in these cases is the relapse in the remaining liver. The reason for this relapse is intrahepatic metastasis by means of the portal vein. In our study, there was no significant difference, in terms of getting staining by Ki-67, between the HCC group and the group with postoperative recurrences in the first year after operation (6 cases) ($p > 0.05$).

In addition 65% of 42 HCC cases in this study were suffering from cirrhosis. However the level of staining was no different between the HCC with cirrhosis and HCC without cirrhosis groups.

With the help of advanced display techniques, early diagnosis of HCC is possible However, diagnosis of HCC by means of display techniques has an effect on the development of HCC in the early period, especially if HBsAg is positive; and in cirrhosis cases. In Japan, a study of this was carried out about this has been done on 28 cases in which tumor size was smaller than 20 mm (5). These tumors are called "small HCC". All these cases were treated with alcohol injection. After this treatment, the 5-year overall survival rates were varied between 60% and 70%. In our study the smallest tumor was 30 mm in diameter. The size was between 40 and 60 mm in 20 cases, and greater than 60 mm in 21 cases. In 23 cases the tumor was disseminated throughout the whole liver. In the light of this, all the cases were considered to be inoperable except for the small one that had been operated on before. There was no significant relationship between staining levels, diameter of tumor, and location of tumor ($p > 0.05$).

There is a significant relationship between Ki-67LI and the degree of differentiation tumor in HCC. In our study 42 cases were stained by Ki-67. Twenty-nine of these were WDCT, 12 PDCT and 1 PLCT. Among these groups, it was found that Ki-67LI was 32.06 ± 29.01 in the well-differentiated group, while it was 60.83 ± 28.74 in the poorly differentiated group. As a result, there was a significant relationship between Ki-67LI and differentiation degree ($t = 2.96$; $p < 0.01$). As a result,

1. Ki-67 can be easily applied in FNAC materials,
2. It can be used to support findings in differentiating reactive hepatocyte and MAC from HCC,
3. There is a significant relationship between differentiation degree and Ki-67LI in HCC cases.

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