

# Computed morphometric analysis and expression of alpha fetoprotein in hepatocellular carcinoma and its related lesion

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## INTRODUCTION

Hepatocellular carcinoma (HCC) is closely related with hepatitis and cirrhosis. In order to investigate the pathogenesis and early pathologic diagnosis of HCC, HCC and related lesions were analyzed qualitatively and quantitatively by automatic image analyser and immunohistochemical assay.

## MATERIALS AND METHODS

### Materials

Specimens obtained from surgical resection, autopsy and needle aspiration biopsy of livers during 1966-1997 were fixed in 10% formalin, embedded in paraffin, made into serial sections, and stained with routine HE. They were divided into seven groups: I. normal liver tissues used as controls (10 cases); II. chronic hepatitis (10 cases); III. chronic hepatitis with early cirrhosis (10 cases); IV. micronodular cirrhosis (13 cases); V. micronodular and macronodular mixed cirrhosis (14 cases); VI. paracancerous cirrhosis (27 cases); VII. HCC (39 cases).

All of the specimens were examined and diagnosed by two pathologists. The diagnosis of hepatitis and cirrhosis was referred to the standard of the Beijing Conference in 1995 and WHO's criteria.

### Morphometry

Thirteen morphometric parameters were determined by the automatic image analyser (Type Q-900, Cambridge Company). And the sections were

enlarged 1000 times under light microscope on the screen of monitor. The cells and nuclei in the sections were traced by light pen. The automatic image analyser was used to determine the nucleus area (NA), the coefficient of variation of NA (NACV), the nucleus perimeter (NP), the nucleus diameter (ND), the roundness of nucleus (NR), the average volume of nucleus (NAV), the cell area (CA), the cell perimeter (CP), the cell diameter (CD), the cell roundness (CR) the average volume of cell (CAV), the ratio of area of the nucleocytoplasm (A-N/C), the ratio of volume of the nucleocytoplasm (V-N/C). After 50-100 cells in each section were examined at random, of the data were analysed by variance (q-test) and stepwise discriminational analysis. Then the equation of discriminational function was set up, the results were compared based on the histopathological classification.

### Immunohistochemical staining

Mouse monoclonal antibody against human AFP and Immunostain S-P Kit were purchased from Fuzhou Maxim Biotechnical Company. Immunostaining of AFP was performed by the S-P method in each case. The procedures of S-P staining were taken according to the manufacturer's recommendations. The color was developed with diaminobenzidine and hematoxylin. Positive and negative controls were simultaneously used to ensure specificity and reliability of the staining.

## RESULTS

### Morphometry

The values of nucleus parameters (NA, NP, ND, NAV, A-N/C and V-N/C) increased gradually and those of cell parameters (CA, CP, CD and CAV) decreased gradually in the sequence of chronic hepatitis, cirrhosis without tumor, paracancerous cirrhosis and HCC. The difference was statistically significant between the group of HCC and the other groups without tumor ( $P < 0.05$ ). The values of NACV, NR and CR in all groups varied irregularly. The values of most parameters (NA, NP, ND, CA, CP, CD, CAV, A-N/C and V-N/C) of the paracancerous cirrhosis were in between those of the cirrhosis without tumor and HCC. The difference was statistically significant ( $P < 0.05$ ). The difference of the value of most parameters was not significant among chronic hepatitis with early cirrhosis, micronodular cirrhosis, mixed

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micronodular and macronodular cirrhosis ( $P<0.05$ ). Results of stepwise discriminational analysis: because the difference of most parameters was not statistically significant among chronic hepatitis with early cirrhosis, micronodular cirrhosis, micronodular and macronodular mixed cirrhosis, the three groups were merged into one cirrhosis group of as a whole. Six of 13 parameters processed by stepwise discriminational analysis were chosen in chronic hepatitis ( $Y_1$ ), cirrhosis without tumor ( $Y_2$ ), paracancerous cirrhosis ( $Y_3$ ) and HCC ( $Y_4$ ). The equation of discriminational function was setup.

$$Y_1 = -526.540 - 32.768(NP) + 631.477(NR) + 6.046(CP) + 1.887(CA) + 19.264(A-N/C) - 0.016(CAV)$$

$$Y_2 = -453.402 - 29.633(NP) + 590.223(NR) + 5.466(CP) + 1.636(CA) + 17.903(A-N/C) - 0.011(CAV)$$

$$Y_3 = -441.556 - 28.851(NP) + 603.816(NR) + 3.875(CP) + 1.854(CA) + 17.991(A-N/C) - 0.014(CAV)$$

$$Y_4 = -623.687 - 36.878(NP) + 698.493(NR) + 5.217(CP) + 2.074(CA) + 23.555(A-N/C) - 0.011(CAV)$$

Fifty-three specimens had been tested, only one of the chronic hepatitis with early cirrhosis was falsely classified into chronic hepatitis and the general conformation rate was 98.2% to pathologic diagnosis.

### Expression of AFP

Immunostaining of AFP was seen in paracancerous cirrhosis and HCC cytoplasms. The positive rates were 33.3% (9/27) and 43.6% (17/39) respectively. The expression of AFP was negative in hepatitis and cirrhosis without tumor. The positive rate of AFP in the paracancerous cirrhosis (33.3%) was significantly higher than in the cirrhosis without tumor (0%, 0/27) ( $P<0.01$ ,  $\chi^2 = 10.8$ ).

### DISCUSSION

HCC which is related with hepatitis and cirrhosis, is one of the common malignant tumors in the world. Popper *et al* considered the occurrence of HCC was a multistep process: HBV infection → persistent inflammation → necrosis → regeneration and repair → hyperplasia → HCC. The course had been studied by morphometry and the results showed that the values of parameters of nucleus increased gradually and those of cells decreased gradually. These suggested that HCC was associated closely with hepatitis and cirrhosis, especially paracancerous cirrhosis.

Paracancerous cirrhosis differed essentially from cirrhosis without tumor. Ren *et al*<sup>[1]</sup> considered the regenerative nodules in the paracancerous cirrhosis had regenerated more actively than those in the cirrhosis without tumor. Watanabe *et al*<sup>[2]</sup> reported that the rate of dysplasia in the paracancerous cirrhosis (25.9%) was higher than that in the cirrhosis without tumor (12%). Dai

*et al*<sup>[3]</sup> reported that some liver cells around the HCC triggered the closed gene to resynthesize AFP. Zhang<sup>[4]</sup> reported that the re presented different degrees positive expression of AFP in the host hepatocytes around cancer and hepatocytes non-neoplastic animals in late stage experimental hepatocarcinoma in rats. All the results showed that the carcinogenesis in paracancerous cirrhosis was more liable to occur than in cirrhosis without tumor. We discovered that values of most parameters in the paracancerous cirrhosis were in between those of the cirrhosis without tumor and HCC by morphometry ( $P<0.05$ ). The expression of AFP was positive in the paracancerous cirrhosis and negative in the cirrhosis without tumor ( $P<0.01$ ). Evidently the paracancerous cirrhosis differed from the cirrhosis without tumor in the respect of function and morphology. It is more likely to be precancerous lesion than cirrhosis without tumor. The values of morphometric parameters in cirrhosis without tumor, regardless of early cirrhosis, micronodular cirrhosis or micronodular and macronodular mixed cirrhosis, were not different significantly. It suggested that the cirrhosis without tumor may be the result of regeneration and repair after HBV infection. Therefore, we inferred that the posthepatitis cirrhosis, the result of persistently affecting HBV, should be evolved into "precancerous cirrhosis" similar to paracancerous cirrhosis, and then some of liver cells were selectively developed into HCC in the circumstance suitable for carcinogenesis. The multisteps in the genesis of HCC may be HBV infection—chronic hepatitis—cirrhosis—"precancerous cirrhosis"—HCC. The paracancerous cirrhosis may be a sequential lesion of precancerous cirrhosis. This inference should be verified by further study. Now, since we can not differentiate the precancerous cirrhosis from the cirrhosis without tumor under light microscope, the precancerous cirrhosis was diagnosed merely by morphometry and immunohistochemical method, which suggested that the patient with precarcinous cirrhosis would probably suffer from HCC in the near future or they had already suffered from HCC somewhere in the liver. It was significant to diagnose HCC as early as possible, especially by using needle aspiration biopsy of liver, thus to decrease the possibility of missing small HCC (SHCC, diameter  $\leq 3$  cm).

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