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Morphometric analysis of hepatocellular carcinoma

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Summary. In order to characterize the cytological features of highly differentiated hepatocellular carcinoma (HCC), a comparative morphometric study was made by observing 30 cases of HCCs and controls (normal, cirrhotic, and atrophic livers). Among trabecular HCCs, normotrabecular subtype (1–2 cell thick cell plate) usually showed minimal cytological atypism and was categorized as well or highly differentiated HCC. Using an image analyzer, the following 4 parameters were applied to quantitate the hepatocyte changes: mean cell size ($\bar{C}$), mean nuclear size ($\bar{N}$), nucleocytoplasmic (N/C) ratio and a coefficient of variance (CV = index of anisokaryosis). In normotrabecular HCCs, $\bar{C}$ was slightly but significantly reduced when compared with normal and cirrhotic livers (t-test: $p<0.005$). The value was further reduced in mid- and macrotrabecular HCCs. Normotrabecular HCCs showed almost the same $\bar{N}$ value as normal and cirrhotic livers but displayed significantly a higher N/C value than those of controls (t-test: $p<0.001$). The N/C ratio became even greater in other types of HCCs. While CV was relatively constant in other HCC groups and controls, it was extremely high in the pleomorphic type of HCC and liver cell dysplasia.

The results indicated that a reduction in $\bar{C}$ and increase in N/C ratio, which appear as "nuclear crowding"in histological specimens, actually occurs in well differentiated HCC. For the histologic diagnosis of well differentiated HCC, it would be very important to examine liver specimens with these observations in mind.

Key words: Hepatoma – Hepatocellular carcinoma – Liver neoplasms – Morphometry – Karyometry

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Introduction

Small nodular lesions occurring in the liver can be easily visualized by the sophisticated methods currently in use for image diagnosis (Okuda 1981; Shinagawa et al. 1984). In fact, an increased number of small hepatocellular carcinomas (HCCs) are now able to be detected during follow-up study of patients with chronic liver diseases. Exact diagnosis, however, may still sometimes be difficult even by histological examination since many of the small HCCs may present a highly differentiated appearance. In our previous studies, several diagnostic points for histological identification of the well differentiated type of small HCC were described. It was indicated that variable nuclear crowding in seemingly normal hepatic cords is one of the most reliable indicators for the diagnosis (Kondo et al. 1986; Kondo et al. 1987). In the present paper, we will describe the results of a morphometric investigation for quantitating the cytological characteristics of such well differentiated HCCs in comparison with those of other types of HCCs or benign hepatic changes.

Materials and methods

Liver specimens, 4 μm thick and stained with haematoxylin and eosin, were selected from our previous autopsy files. They included 30 cases of HCC, 5 normal livers, 10 cirrhotic livers, and 5 livers containing atrophic areas. The HCC cases, all of which were associated with liver cirrhosis, comprised trabecular (Figs. 1A–1C), pseudoglandular, pleomorphic (Fig. 1D), and anaplastic (Fig. 1E) types. According to the thickness of tumour cell trabeculae, the trabecular type was further divided into normotrabecular (1–2 cells thick, Fig. 1A), midtrabecular (3–7 cells thick, Fig. 1B) and macrotrabecular (more than 8 cells thick, Fig. 1C) subtypes. The normotrabecular type usually presented a well differentiated cytological appearance, being

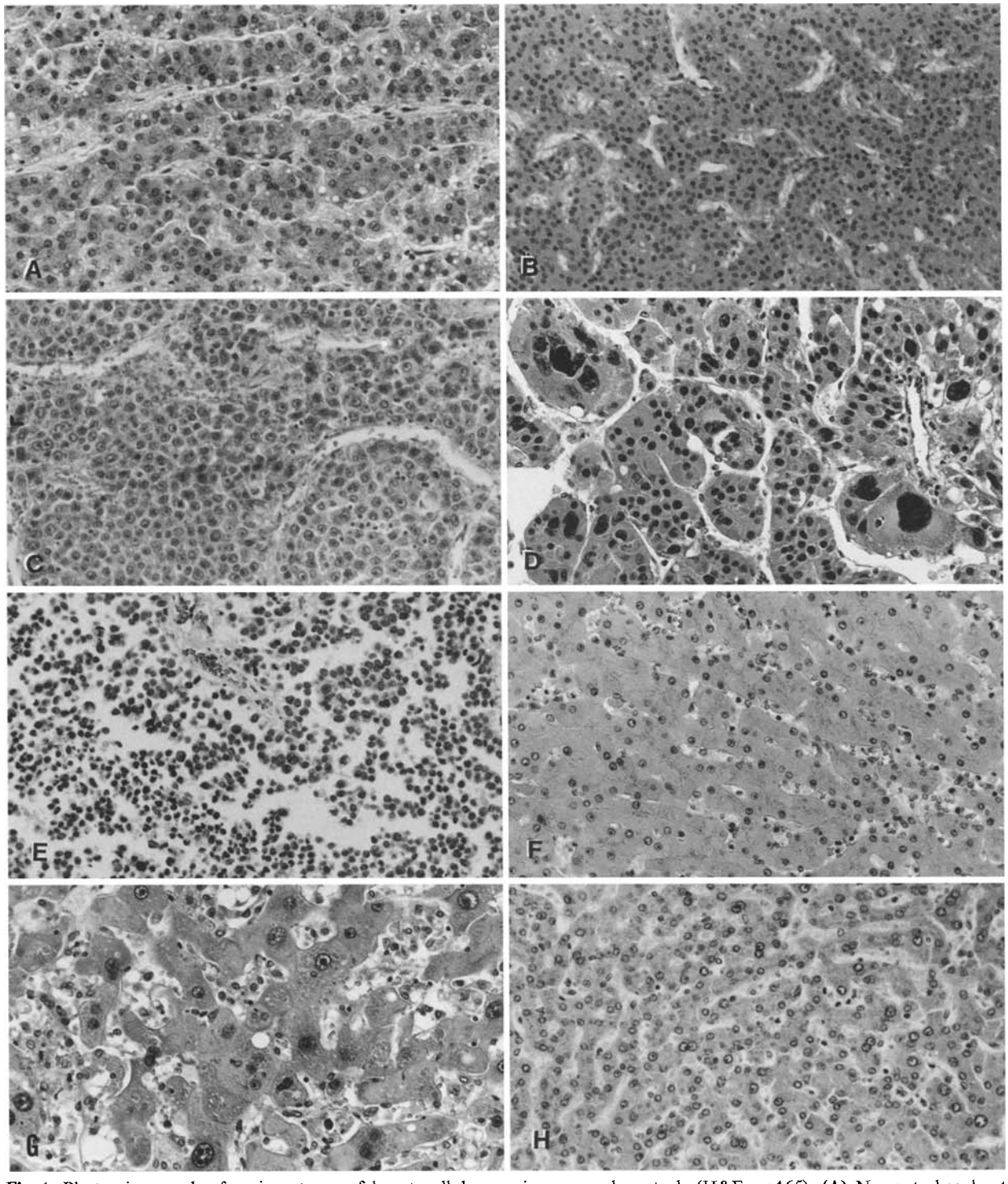

Fig. 1. Photomicrograph of various types of hepatocellular carcinomas and controls (H&E, ×165). **(A)** Normotrabecular type consisting of hepatic cords with almost normal thickness (1–2 cells thick). Note some nuclear crowding resulting in increase of N/C ratio which can be easily recognizable when compared with liver cirrhosis **(F)**. Though nuclear atypism is minimal, this tumour showed distinct extracapsular invasion. **(B)** Midtrabecular type. Trabecular thickening and nuclear crowding become more prominent. Cell size is smaller than normotrabecular type. **(C)** Macrotrabecular type characterized by formation of large plump cell cords. **(D)** Pleomorphic type showing marked cellular and nuclear atypism. Anisokaryosis is remarkable. **(E)** Anaplastic type presenting extremely high N/C ratio. This pattern was observed as a focal variation of a tumor which was mostly consisted of typical trabecular type of hepatocellular carcinoma. **(F)** Liver cirrhosis (ordinary pseudolobule). Nuclei are rather loosely scattered within cell cords. **(G)** Liver cell dysplasia. Cell as well as nuclear size is very large and anisokaryosis is prominent. **(H)** Liver cell atrophy. Significant nuclear crowding due to reduction in cell size with increase of N/C ratio

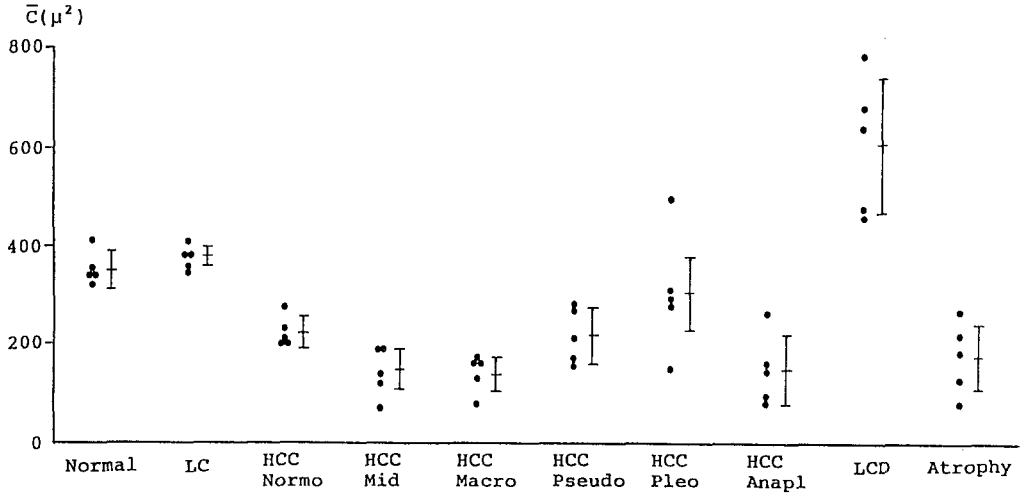

Fig. 2. Mean cell size ($\bar{C}$). Point shows $\bar{C}$ of each case and bars show mean and standard deviations of each group. *LC*=liver cirrhosis; *HCC*=hepatocellular carcinoma; *Normo*=normotrabecular; *Mid*=midtrabecular; *Macro*=macrotrabecular; *Pseudo*=pseudoglandular; *Pleo*=pleomorphic; *Anapl*=anaplastic; *LCD*=liver cell dysplasia

categorized as grade I of Edmondson and Steiner's classification (1954). The midtrabecular type was compatible with the microtrabecular type of Peters' classification (1976). Included in the cases of liver cirrhosis were ordinary pseudolobules (Fig. 1 F) and those containing an area of "liver cell dysplasia" (Fig. 1 G) (Anthony 1976; Anthony et al. 1973). Each group consisted of 5 cases. Causative factors were suggested in 32 cases of liver cirrhosis with or without HCC, comprising hepatitis B virus infection in 19, alcoholic abuse in 10, and blood transfusion in 3. Areas of liver cell atrophy were selected from 3 livers with metastatic carcinoma and 2 with cardiac insufficiency.

The histologic specimens thus prepared were photographed, excluding necrotic or other changes unsuitable for morphometry, and enlarged to 500 times in the final printing. The areas photographed were arbitrarily selected to contain each of the above mentioned histological patterns, without any specific consideration regarding tumour size or prevalent tumour histology. The magnification (×500) was input to an image analyzing program (MGX-1000, Muto Electric, Tokyo, Japan) which used a digitizer (ID, Muto Electric) coupled to a computer (CX-1, Canon, Tokyo). The photographs were placed on a digitizer table and several trabecular areas containing 10 to 30 nuclei were outlined. In the anaplastic type of HCC, the trabeculae tended to dissociate so that many small cell clusters were carefully selected to exclude those undergoing regressive changes. The total trabecular areas thus examined contained more then 100 nuclei. Subsequently the nuclear outline of each nucleus included in the trabecular areas was traced.

By tracing the photographs, individual nuclear size, mean nuclear size ($\bar{N}$), coefficient of variance of nuclei (CV), and trabecular area could be automatically displayed according to the image analyzing system program. Mean cell size ($\bar{C}$) was estimated as mean trabecular area shared by one nucleus and presented by following calculation:

$$\bar{C}\ (\mu^2)=\frac{\text{trabecular area } (\mu^2)}{\text{number of nuclei contained}}$$

Although this $\bar{C}$ value is not equivalent to true mean cell size calculated by measuring individual tumour cells, it may still be an excellent parameter for displaying the grade of "nuclear crowding".

Nucleocytoplasmic ratio (N/C) was calculated as follows:

$$\text{N/C } (\%)=\frac{\text{mean nuclear size } (\bar{N})}{\text{mean cell size } (\bar{C})}\times 100$$

In the present study, statistical analysis was performed using the *t*-test.

Results

For the mean cell size Fig. 2 shows the $\bar{C}$ of each case (point) and mean value of $\bar{C}$ ($\pm$SD) of each group. As shown in Fig. 2, $\bar{C}$ was mostly distributed between 300 μ^2 and 400 μ^2 in normal and cirrhotic livers, the value being larger than that of normotrabecular HCC. It was thus clarified that in the normotrabecular HCC, the cell size was slightly but significantly reduced ($p<0.005$) (Fig. 1 A). The value was further reduced in mid- and macrotrabecular HCCs (normo- vs. mid- or macrotrabecular HCC, $p<0.02$), indicating that all of the trabecular HCCs so far examined had a $\bar{C}$ value of less than 300 μ^2 (Fig. 2). Liver cell atrophy (Fig. 1 H) showed a similar distribution pattern to these HCC groups.

The pleomorphic type of HCC was composed of tumour cells of variable size. It was not uncommon to find that many small cells were present among the large tumour cells including bizarre multinucleated giant cells (Fig. 1 D). Accordingly the $\bar{C}$ value could sometimes be smaller than that of normal or cirrhotic livers (Fig. 2). Pleomorphic HCC, however, tended to present a somewhat higher $\bar{C}$ value when compared with ordinary trabecular HCCs. Cellular enlargement was quite outstanding in liver cell dysplasia (Fig. 1 G), whereas

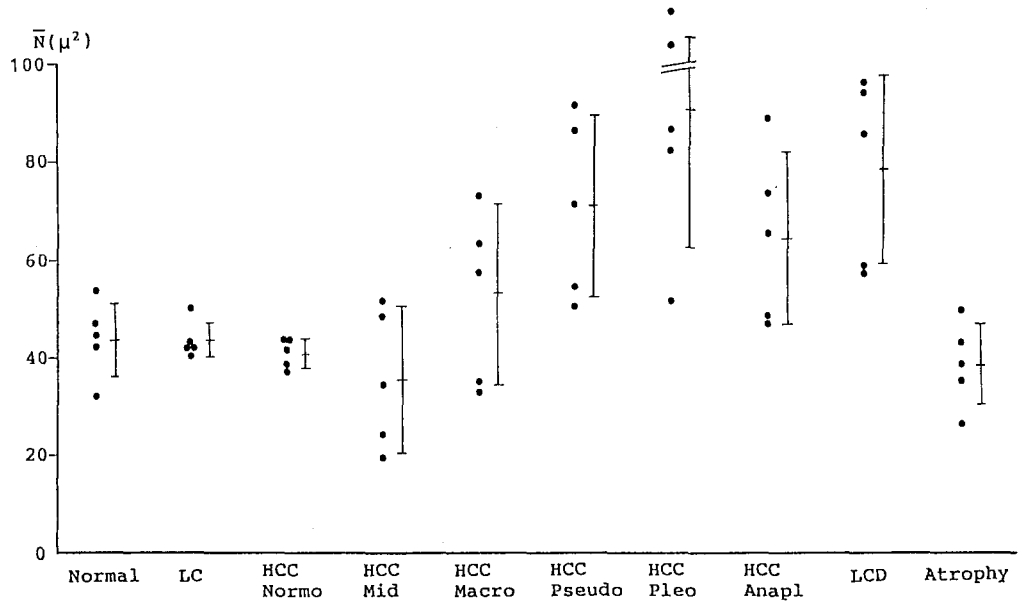

Fig. 3. Mean nuclear size ($\bar{N}$)

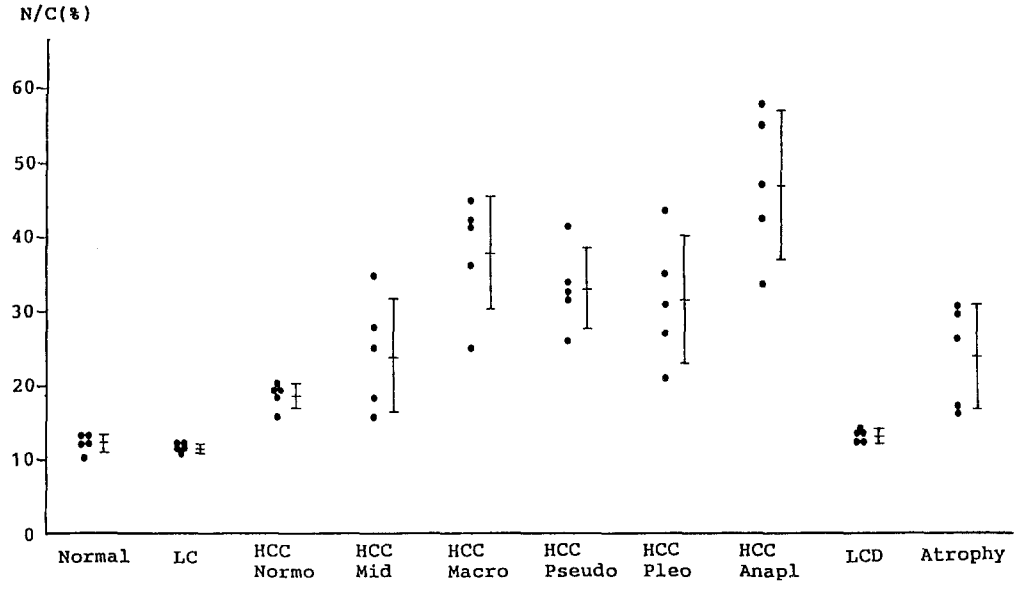

Fig. 4. Nucleocytoplasmic ratio (N/C)

anaplastic type of HCC (Fig. 1 E) was made up of small cells.

Mean nuclear size ($\bar{N}$) mostly ranged from 30 μ^2 to 50 μ^2 in normal as well as cirrhotic livers and in normotrabecular HCC (Fig. 3). Midtrabecular HCC (Fig. 1 B) had rather smaller nuclei than normotrabecular HCC, although the difference was not statistically significant. $\bar{N}$ of liver cell atrophy resembled that of normo- and midtrabecular HCCs. Pseudoglandular, and anaplastic types of HCC had much larger nuclei, as did liver cell dysplasia. Among the various types of HCCs, the pleomorphic type showed the highest $\bar{N}$ value.

In the present study, all of the HCC cases showed a nucleocytoplasmic ratio (N/C ratio) of more than 15% while most of the cases with normal or cirrhotic livers were less than 15% (Fig. 4). The differences between normotrabecular HCC and normal or cirrhotic livers were statistically significant ($p<0.001$). Among the HCC cases, normotrabecular and midtrabecular types displayed the lowest value and anaplastic type the highest.

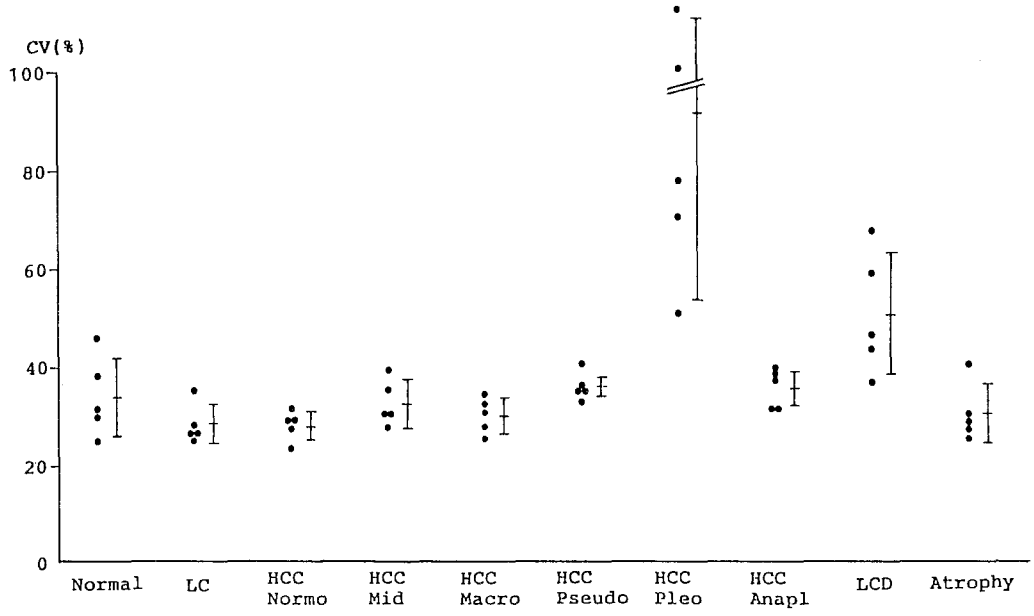

Fig. 5. Coefficient of variance (*CV*)

Other types of HCCs had a N/C ratio between these two extremes. Liver cell dysplasia showed almost the same value as normal or cirrhotic livers while atrophic hepatocytes resembled normo- or midtrabecular HCC.

The pleomorphic type of HCC showed an extremely high coefficient of variance (VC), followed by liver cell dysplasia (Fig. 5). Pseudoglandular and anaplastic HCCs showed slightly elevated CV. The value did not differ greatly among normal livers, liver cirrhosis, normotrabecular HCC, and midtrabecular HCC.

Discussion

By analogy with many other carcinomas, one might assume that distinctive cytological atypia becomes manifest during hepatocarcinogenesis in humans. A close association between the occurrence of HCC and the appearance of atypical cells has been noted, which is referred to as liver cell dysplasia (LCD) (Anthony 1976; Anthony et al. 1973). Yet it seems that there is no conclusive evidence to indicate the premalignant nature of LCD. Conversely, small, developing HCC nodules may be composed of hepatic cords with minimal, if any, atypical changes, being compatible with grade I HCC as evaluated by Edmondson and Steiner's classification (1954). The grade I HCC, however, is not quantitatively distinct in terms of nuclear atypism from normal hepatocytes (Hemmi 1983), indicating that additional criteria are needed for correct diagnosis. In such a well differentiated HCC, an increase in nuclear number was frequently observed while the finding was rare in benign liver tissue. (Kondo et al. 1986; Kondo et al. 1987). Morphometric analysis may be useful for quantitative evaluation of this finding. Quantitative cytological studies have previously been carried out with HCCs (Tezuka et al. 1983; Watanabe et al. 1983) and the data thus far presented are indeed important for revealing the general features of HCCs, but not for characterizing well differentiated HCC.

In the present study, we classified the trabecular type of HCCs into three subtypes based on trabecular thickness, and carried out a morphometric analysis accordingly. In the normotrabecular type of HCC, cellular and structural atypism was very mild (consistent with grade I HCC). Regardless of the tumour size, $\bar{N}$ was compatible with controls (normal liver and liver cirrhosis). By contrast, mean cell size was slightly but significantly smaller than controls ($p < 0.005$), leading to an apparent increase in the N/C ratio. Coefficient of variance, an index of anisokaryosis, was similar to that of controls. It was thus clearly shown that nuclear crowding indeed occurred in the normotrabecular, well differentiated type of HCC.

The reduction in cell size became prominent in accordance with progressive thickening of tumour cell cords, resulting in a further increase in the N/C ratio. In pseudoglandular, pleomorphic (grade III HCC) and anaplastic (grade IV HCC) types, an increase of N/C ratio was mostly caused by an enlargement of nuclear size. Interestingly,

it appears that there were sequential steps in regard to increase of N/C ratio, ranging from controls to normotrabecular, midtrabecular, macrotrabecular, pleomorphic, and anaplastic HCCs (Fig. 4).

In view of its cytological and structural resemblance to normal hepatic cords and its prevalence in minute tumour nodules (Kondo et al. 1986; Kondo et al. 1987), the normotrabecular subtype could be a prototype of early developing HCC. It is suggested that the precancerous lesion, if present, becomes manifest as an intermediate alteration between common hepatic cords and the normotrabecular HCC. Some investigators have noticed an area consisting of small hepatocytes as a possible precancerous lesion (Altmann 1977; Tezuka et al. 1983; Watanabe et al. 1983). In addition, experimentally induced precancerous foci may contain hepatocytes with reduced cell size (Bannasch 1976; Kohen et al. 1983). Although the exact nature of these small hepatocytes is still unknown, these results seem to be in general accord with ours. From the morphometric aspects, LCD somewhat resembled pleomorphic HCC but was quite distinct from normotrabecular HCC. It is unlikely that this lesion represents liver cell alteration in the premalignant stage.

We are now examining a number of liver biopsy specimens obtained from patients with various liver diseases, and in particular those of nodular hepatic lesions. Apart from overt large HCC nodules, some minute HCC nodules or borderline lesions are encountered among these hepatic samples. In fact, in the patients with liver cirrhosis the most important issue is how to distinguish highly differentiated HCC from cirrhotic parenchyma.

We have been able to define reliable criteria for this distinction by observing defined autopsy cases of HCCs. Two items, changes in cell size and N/C ratio, which become manifest as "nuclear crowding" in histological specimens, were thus shown to be most reliable indicators for the distinction between well differentiated HCC and liver cirrhosis. In routine histological observations, this evaluation should be made by comparing at least two samples that comprise nodular tissue as well as extranodular parenchyma, since the changes may sometimes be subtle. This should also be recommended in the case of biopsy examinations. In addition to the "nuclear crowding", other noticeable findings, notably increased cytoplasmic basophilia and microacinar formation may also be helpful for the histological diagnosis of small HCC nodules as reported previously (Kondo et al. 1986; Kondo et al. 1987).

Another finding of interest is that atrophic hepatocytes presented similar morphometric values to those of well differentiated HCCs, though overall histological appearances could be distinguished. Other types of HCCs also presented definite morphometric characteristics. The data, however, may not be so important for practical application since these HCCs are easily diagnosed from their outstanding histological features.

Various benign hepatic tumours, tumour-like conditions, or borderline lesions were not included in the present study. Similar morphometric examinations of those lesions would also seem to be useful for a more comprehensive understanding of early HCC.

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