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Original Article

Pathologic features of small hepatocellular carcinoma: analysis of 61 cases

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Abstract: Small hepatocellular carcinoma (HCC) is defined as hepatocellular carcinoma measuring less than 2 cm in diameter. Sixty-one cases of small HCC were analyzed to investigate the pathologic features of small HCC. Sixty-one cases of small HCC were selected from HCC cases which underwent surgical resection at Yeungnam University Hospital. The pathologic findings were analyzed. There were 48 male patients and 13 female patients, with ages ranging from 37 to 75 years (median, 57 years). Hepatitis B virus infection was present in 75.4% (46/61), hepatitis C virus infection in 11.4% (7/61), and alcoholic liver disease in 6.6% (4/61). Cirrhosis was present in 77.0% (47/61). Grossly, vaguely nodular type was present in 16.4% (10/61) and distinctly nodular type in 83.6% (51/61). Histologically, well differentiated type was present in 62.3% (38/61), moderately differentiated type in 36.1% (22/61), and poorly differentiated type in 1.6% (1/61). Vascular invasion was present in 8.2% (5/61). In the non-neoplastic liver, high grade dysplastic nodule was present in 16.4% (10/61). In conclusion, a large percentage of small HCCs are well differentiated. The majority of small HCCs arise in cirrhotic liver. Hepatitis B virus infection is the most common cause of small HCCs in Korea.

Keywords: Small hepatocellular carcinoma, hepatitis B, liver cirrhosis, pathology

Introduction

Hepatocellular carcinoma (HCC) is a common malignant tumor and many people in Korea as well as the rest of the world suffer from this tumor. Advances in imaging techniques and establishment of surveillance protocols for high-risk populations have led to the early detection of small hepatic nodules in patients with chronic liver diseases [1]. The increase in the number of resected liver specimens has provided pathologists more opportunity to examine earlier HCC lesions [2]. The International Working Party (IWP) for nodular hepatocellular lesions defined small HCC as a tumor measuring less than 2 cm [3]. Small HCC is classified according to two major types: the vaguely nodular and distinctly nodular type [4]. Vaguely nodular small HCC corresponds to early HCC [5]. To date few studies providing pathologic data on small HCC have been reported [6-9].

In the current study, we studied 61 surgically resected small HCCs in order to clarify the pathologic features of small HCC.

Materials and methods

Patients and specimen

Sixty-one cases were selected from HCC cases which underwent surgical resection between 2000 and 2008 at Yeungnam University Hospital. All tumors measured less than 2 cm in diameter. The resected liver specimens were fixed in 10% formalin. The histology of the tumorous and nontumorous portions was studied by hematoxylin-eosin, periodic acid-Schiff, reticulin, and trichrome stain. Light microscopic examination was performed. Macroscopic types were classified according to five types based on Liver Cancer Study Group of Japan criteria: 1) vaguely nodular type (indistinct margins), 2) distinctly nodular type (distinct margins), including simple nodular type, simple

Table 1. Clinicopathologic features in small hepatocellular carcinoma

Variables	Small HCC (n=61)
Age (years)	37-75 (median, 57)
Sex	
Male	48 (78.7%)
Female	13 (21.3%)
Underlying diseases	
HBV	46 (75.4%)
HCV	7 (11.4%)
Alcoholic	4 (6.6%)
Unknown	4 (6.6%)
Cirrhosis	
Present	47 (77.0%)
Absent	14 (23.0%)
Change	
Large cell change	20 (32.8%)
Small cell change	15 (24.6%)
Dysplastic nodule	
Low grade dysplastic nodule	8 (13.1%)
High grade dysplastic nodule	10 (16.4%)
Fatty change in tumor	
Present	15 (24.6%)
Absent	46 (75.4%)
Vascular invasion	
Present	5 (8.2%)
Absent	56 (91.8%)

nodular type with extranodular growth, multinodular confluent type, and 3) infiltrative type [10]. Histological grading of HCC was classified on the base of tumor differentiation: well-differentiated, moderately differentiated, poorly differentiated, and undifferentiated according to WHO criteria [4]. Pathologic reports were reviewed. This study was approved by the institutional review board of Yeungnam University hospital (YUH-2015-04-014).

Statistical analysis

The Fisher exact test and Mann-Whitney test were used for determination of correlation between gross types and clinicopathologic variables in small hepatocellular carcinoma. Survival rates between gross types were calculated by the Kaplan-Meier method, and statistical significance between curves was tested using the Breslow test. A *P* value of less than .05 was considered statistically significant. SPSS (version 18.0 for Windows; SPSS Inc., Chicago, IL) was used for statistical analysis.

Results

Clinical findings

There were 48 male patients and 13 female patients, with ages ranging from 37 to 75 years (median, 57 years) (**Table 1**). Hepatitis B virus infection was present in 75.4% (46/61), hepatitis C virus infection in 11.4% (7/61), alcoholic liver disease in 6.6% (4/61), and unknown cause in 6.6% (4/61). Preoperative radiologic findings were available in 57 patients. Radiologic impressions were hepatocellular carcinoma in 54 patients, dysplastic nodule in 2 patients, and cholangiocarcinoma in 1 patient, respectively.

Pathological findings

Grossly, vaguely nodular type was present in 16.4% (10/61) (**Figure 1A** and **1B**) and distinctly nodular type in 86.6% (51/61), including simple nodular type (35/61) (**Figure 1C** and **1D**), extranodular extension type (14/61) (**Figure 2A** and **2B**), and multinodular confluent type (2/51) (**Table 2**). Nodule in nodule appearance was present in 9 patients (14.8%) (**Figure 2C** and **2D**). Fifty five patients (90.2%) had only one tumor mass. Six patients (9.8%) had more than one tumor mass and the largest mass was less than 2 cm. Histologically, 62.3% (38/61) were well differentiated type. 36.1% (22/61) were moderately differentiated type, and 1.6% (1/61) were poorly differentiated type. Vaguely nodular HCCs showed a thin trabecular pattern and no significant obvious cytological atypia. Comparison of vaguely nodular small HCC and distinctly nodular small HCC is shown in **Table 3**. The mean age of patients with vaguely nodular type was 54.8 ± 8.79 (mean ± SD). The mean age of patients with distinctly nodular type was 57.6 ± 10.88. The vaguely nodular small HCCs had a mean diameter of 1.37 ± 0.41 cm, whereas distinctly nodular small HCCs had a mean diameter of 1.54 ± 0.40 cm. In the vaguely nodular small HCCs, the tumor cells were well differentiated in 70% (7/10) (**Figure 3A-D**) and moderately differentiated in 30% (3/10). In the distinctly nodular small HCCs, the tumor cells were well differentiated in 60.8% (31/51), moderately differentiated in 37.3% (19/51) (**Figure 4A-C**), and poorly differentiated in 1.9% (1/51) (**Figure 4D**). Fatty change within tumors was present in 24.6% (15/61). Vascular invasion was present in 8.2% (5/61). Vascular invasion was found only in distinctly nodular

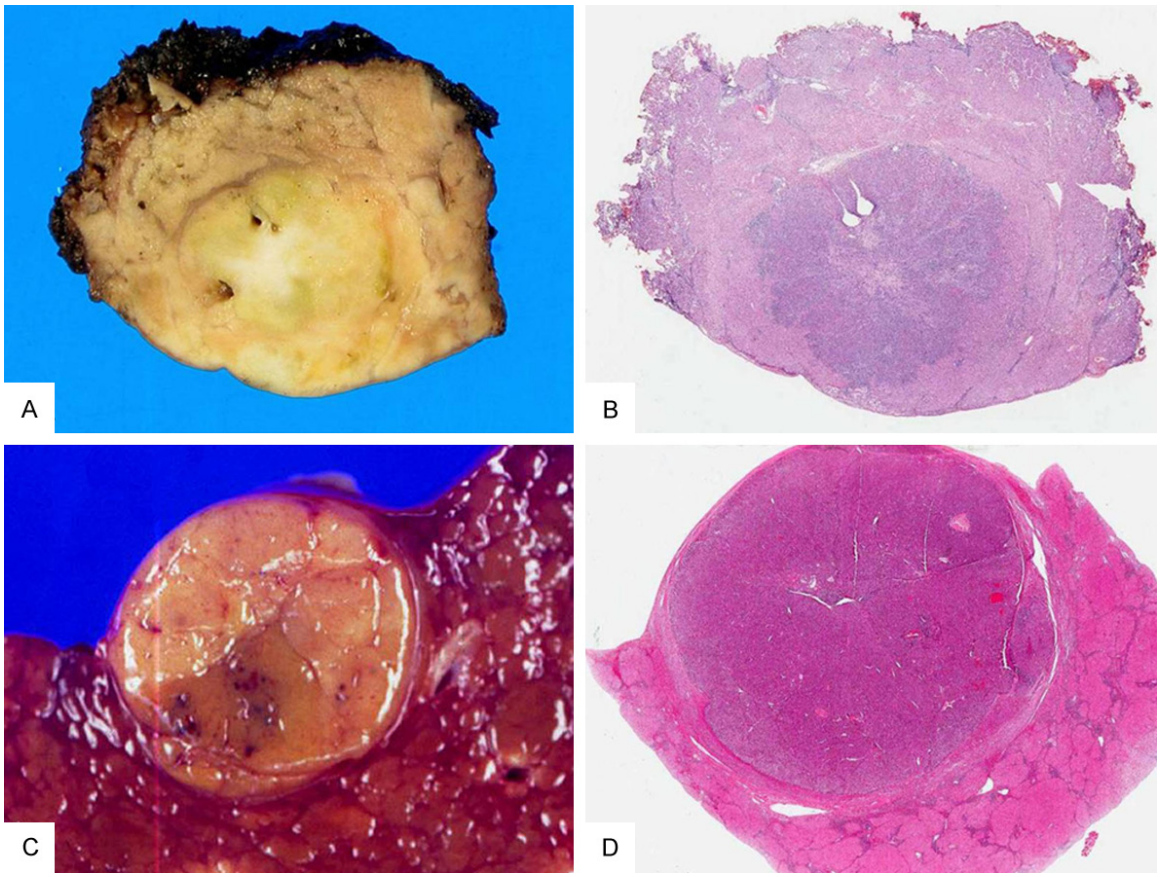

Figure 1. Macroscopic findings of small HCC. A, B. Vaguely nodular HCC. The demarcation between the tumor and nontumor is not distinct. C, D. Distinctly nodular HCC, simple nodular type. The demarcation between the tumor and nontumor is distinct.

type. Cirrhosis was present in 77.0% (47/61). No statistically significant correlation was observed between gross type and age, tumor size, histologic grade, vascular invasion, fatty change, and cirrhosis, respectively ($P > 0.05$).

In the non-neoplastic liver, large cell change was present in 32.8% (20/61) and small cell change was present in 24.6% (15/61). Low grade dysplastic nodule was present in 13.1% (8/61), and high grade dysplastic nodule was present in 16.4% (10/61).

Survival of small hepatocellular carcinoma patients based on gross type

There was no significant difference in overall survival between patients with vaguely nodular type and patients with distinctly nodular type (P=0.694) (**Figure 5**).

Discussion

Worldwide, hepatocellular carcinoma (HCC) is the fifth leading cause of death in males and accounts for approximately 5.4% of all cancers, although its incidence varies widely in different parts of the world [11]. The highest incidences of HCC are found in Asian countries (southeast China, Korea, Taiwan) and sub-Saharan African countries. The main etiologic agents for HCC are chronic hepatitis B and C, alcoholic cirrhosis, non-alcoholic fatty liver disease, and hemochromatosis. The chronic inflammation and cellular regeneration associated with viral hepatitis or activation of the IL-6/JAK/STAT pathway may be predisposing factors for development of HCC [11]. In the current study, chronic hepatitis B was the most common cause of small HCC in Korea, accounting for approximately 75% of cases. Liver cirrhosis was found in 77% of cases. Liver cirrhosis is known as a major clinical risk factor for HCC [4].

There is a pronounced male preponderance throughout the world, about 3:1 in low-incidence areas and as high as 8:1 in high incidence areas [11]. In the current study, male:

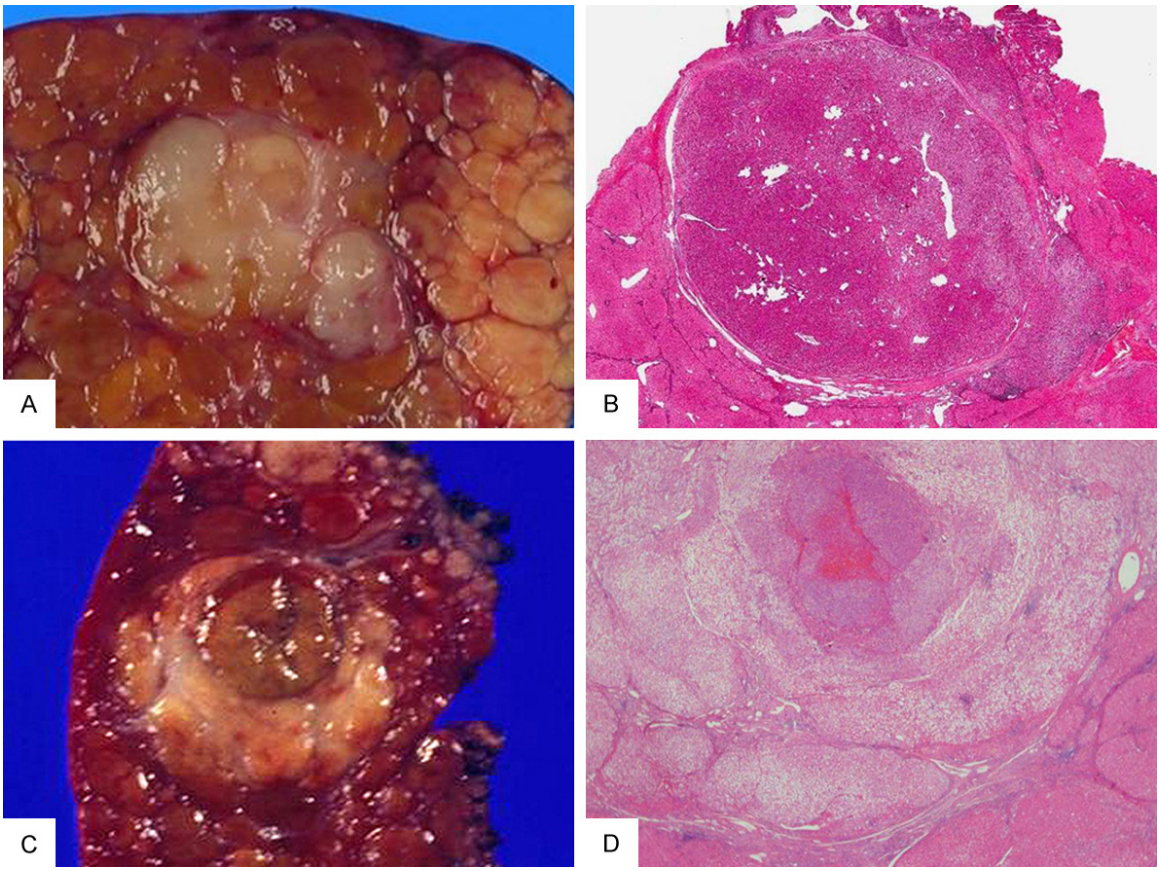

Figure 2. Macroscopic findings of small HCC. A, B. Distinctly nodular HCC, simple nodular type with extranodular growth. Extranodular growth is present. C, D. Distinctly nodular HCC, simple nodular type. Nodule in nodule appearance is present.

Table 2. Macroscopic type in small hepatocellular carcinoma

Macroscopic types	Small HCC
Vaguely nodular type	10 (16.4%)
Distinctly nodular type	
Simple nodular	35 (57.4%)
Extranodular extension	14 (22.9%)
Multinodular confluent	2 (3.3%)
Total	61 (100%)

female ratio was 3.69:1. The reason for the gender imbalance is not known. The median age was 57.

The evolution of dysplastic nodules to hepatocellular carcinoma within several months to a few years of follow-up is well documented [8]. Vaguely nodular HCC has been identified as early well-differentiated neoplasm that usually measures less than 1.5 cm in greatest dimension. As this lesion grows larger, it may transform into nodular HCC with a distinct margin [8]. In the current study, the distinctly nodular type tended to be larger than the vaguely nodular type. In addition, the age of patients with distinctly nodular type tended to older compared with patients with vaguely nodular type.

Small HCC is defined as HCC measuring less than 2 cm in diameter. More recent studies support the division of small HCC into two clinicopathological groups, termed early HCC and progressed HCC [4]. Well-differentiated small HCC of the vaguely nodular type appears to be early stage of HCC development and small HCCs of the distinctly nodular type represent progressed carcinoma [5]. In the current study, vaguely nodular HCCs were mostly well differentiated and had no vascular invasion. Distinctly nodular HCCs were moderately differentiated in 37.3% of cases and had vascular invasion. Early HCC has a longer time to recurrence and a higher 5-year survival rate com-

Table 3. Comparison of vaguely nodular type and distinctly nodular type in small hepatocellular carcinoma

Variables	Vaguely nodular (n=10)	Distinctly nodular (n=51)	*P*
Age (years) (mean ± SD)	54.8 ± 8.79	57.6 ± 10.88	0.320
Tumor size (mean ± SD)	1.37 ± 0.41 cm	1.54 ± 0.40 cm	0.1987
Histologic grade			0.776
Well differentiated	7 (70%)	31 (60.8%)	
Moderately differentiated	3 (30%)	19 (37.3%)	
Poorly differentiated	0 (0%)	1 (1.9%)	
Vascular invasion	0 (0%)	5 (9.8%)	0.580
Fatty change	3 (30%)	12 (23.5%)	0.696
Cirrhosis	7 (70%)	40 (78%)	0.683

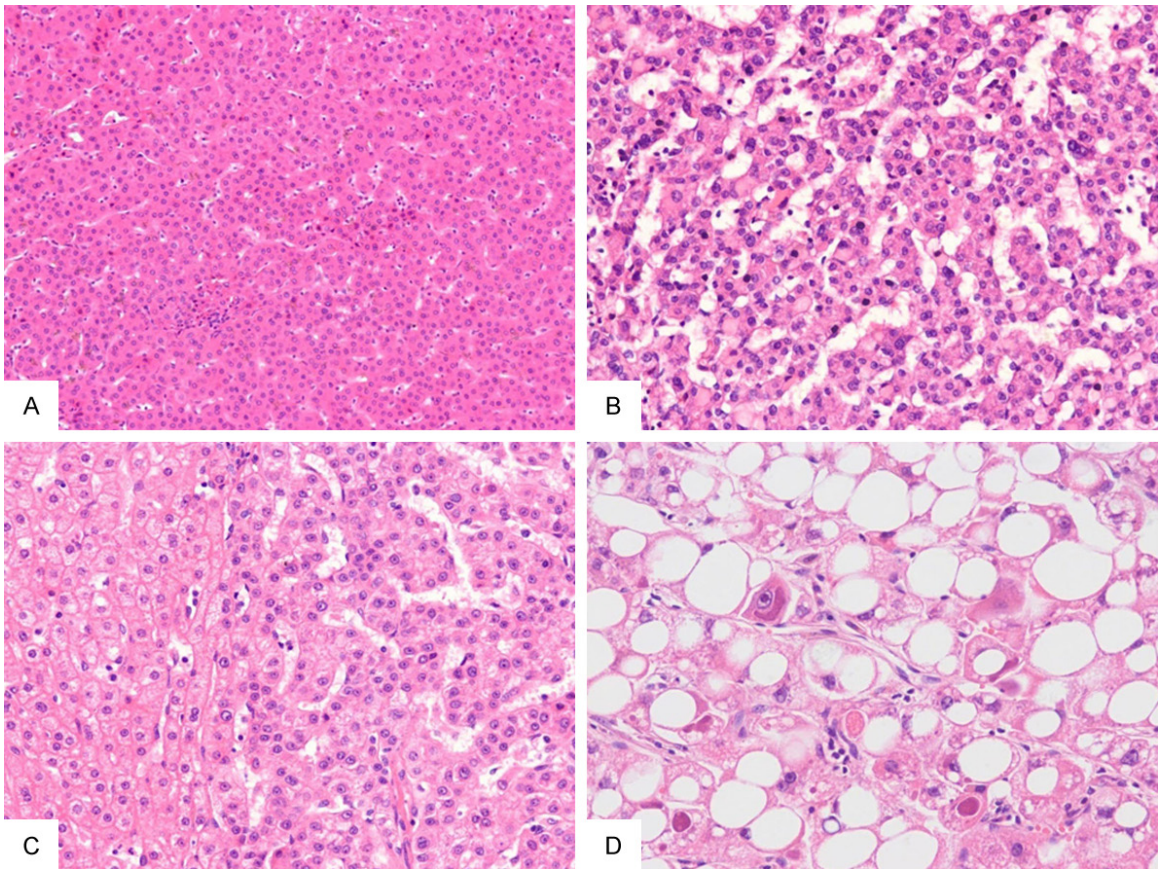

Figure 3. Microscopic findings of vaguely nodular HCC. A. Well differentiated HCC. Well differentiated tumor cells are present (hematoxylin-eosin stain, x40). B. Well differentiated HCC. The thin trabecular pattern is present (hematoxylin-eosin stain, x100). C. Well differentiated HCC. Replacing growth pattern and no capsule are present (hematoxylin-eosin stain, x200). D. Well differentiated HCC. Fatty change is present (hematoxylin-eosin stain, x200).

pared with progressed HCC [12]. In the present study, patients with vaguely nodular type had no significantly better overall survival than those with distinctly nodular type. Our study is limited by relatively small sample size. It is needed to investigate further.

Small cell change, large cell change, low grade dysplastic nodule and high grade dysplastic nodule have been identified as precursor lesions to HCC. HBV-related large cell change is more consistent with dysplastic rather than merely reactive hepatocytes [13]. Small cell

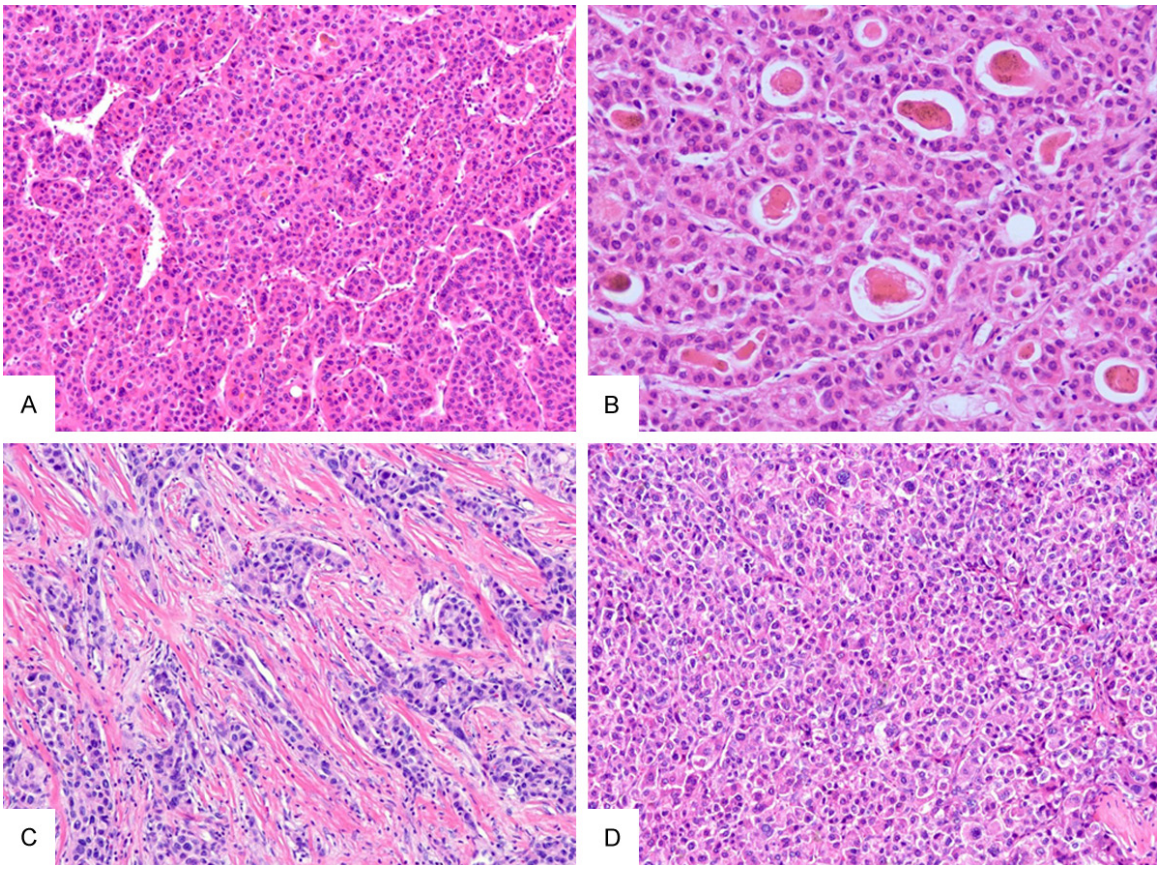

Figure 4. Microscopic findings of distinctly nodular HCC. A. Moderately differentiated HCC. The tumor cells are arranged in a trabecular pattern (hematoxylin-eosin stain, x100). B. Moderately differentiated HCC. The tumor cells are arranged in a pseudoglandular pattern (hematoxylin-eosin stain, x100). C. Moderately differentiated HCC. Sinusoidal blood vessels are replaced by fibrous connective tissue (hematoxylin-eosin stain, x100). D. Poorly differentiated HCC. The tumor cells show compact growth (hematoxylin-eosin stain, x100).

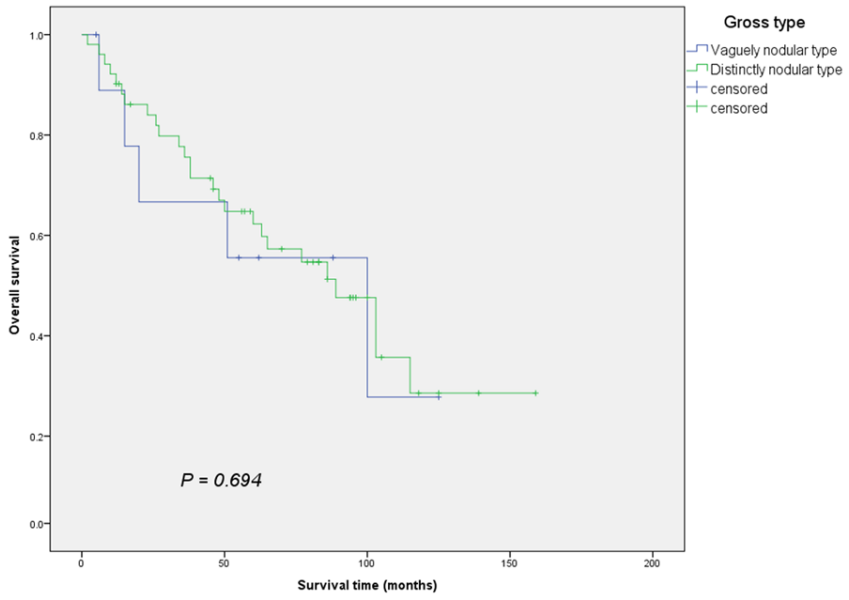

Figure 5. Kaplan-Meier curve demonstrates that overall survival is not significantly better in vaguely nodular type compared with distinctly nodular type.

change is considered to be a more advanced precursor lesion than large cell change [14]. Dysplastic nodules are classified as low and high grade according to the degree of atypia [3]. High grade dysplastic nodule is the most advanced precancerous lesion of the liver [15]. Dysplastic nodules may evolve over time to vaguely nodular HCC, which may then transform into distinctly nodular HCC. In the current study, 15% of cases showed small cell

change in the nontumorous liver and 16.4% of cases showed high grade dysplastic nodule in the nontumorous liver.

With advances in diagnostic imaging, detection of HCCs less than 2 cm in diameter has increased. Detection of premalignant lesions of HCC is important. Diagnosis of early HCC can improve patient survival. Histologically, it is sometimes difficult to distinguish early HCC from premalignant lesions. In recent studies, glypican-3, heat shock protein 70 and glutamine synthetase could be marker for diagnosis of early HCC [16, 17]. The diagnostic accuracy of this panel of markers has been tested in liver biopsy of hepatocellular nodules. Correlation of the histological features with clinical and radiological findings is important for accurate diagnosis.

Fatty change has been reported in premalignant nodules and early HCC [18]. Hypoxic condition or hypoperfusion in the tumor has been proposed as a possible mechanism of fatty change. In the current study, fatty change of tumor cells was found in 30% of vaguely nodular type and 23.5% of distinctly nodular type, respectively. The prevalence of fatty change decreases along with increasing tumor size [5].

When a well differentiated HCC reaches a size of approximately 1.0-1.5 cm, less differentiated cancerous tissue evolve within it [19]. Such a phenomenon is often appreciated grossly and histologically as a nodule in nodule appearance. In the current study, 14.8% of HCCs were nodule in nodule appearance. Regarding morphologic evolution of HCC, HCCs arise either from premalignant lesions such as dysplastic nodule in liver cirrhosis or de novo lesions in noncirrhotic liver [20]. HCC is well differentiated in the early stage, when it grows slowly. The growth rate accelerates when dedifferentiation occurs, with or without a nodular in nodule appearance, and the tumor develops to advanced HCC. In this study, multicentricity was present in 9.8% of cases. Multicentric development is relatively common in HCC [21].

In conclusion, a large percentage of small HCCs are well differentiated. The majority of small HCCs arise in cirrhotic liver. Hepatitis B virus infection is the most common cause of small HCCs in Korea. Further studies on molecular markers for precise diagnosis for early HCC and mechanisms of hepatocarcinogensis are required.

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Disclosure of conflict of interest

None.

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