RESEARCH COMMUNICATION

# Histologic Subtypes of Hepatocellular Carcinoma in the Southern Thai Population

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## Abstract

**Since there has been no report on histologic subtypes of hepatocellular carcinoma (HCC) and its significance in the Thai population, the present study was conducted to elucidate the situation through appraisal of histologic and laboratory records. A total of 180 archived microscopic slides of HCC in Sonklanagarind Hospital from 1991 to 1998 were of good enough quality with sufficient tissue to be reviewed. The reclassified histologic subtypes were correlated with microscopic features and laboratory data. Of the 180 cases, 147 were males and hepatitis B was the main etiologic factor. The histologic subtypes of HCC were trabecular 63.3%, compact 15.6%, scirrhous 7.8%, pseudoglandular 5%, and fibrolamellar 0.6%. There was no correlation between histologic subtypes and morphological findings, as well as HBV, HCV, and cirrhotic status. A correlation between AFP levels and the AST/ALT ratio was evident.**

**Key Words**: hepatocellular carcinoma - histologic subtype

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## Introduction

Hepatocellular carcinoma (HCC) is the most common liver cancer in Songkhla province in the south of Thailand. The age-standardized incidence rate of liver cancer in the area during 1992-1994 was 14.2 per 100,000 population in males and 4.6 in females (Deerassamee et al., 1999). Guided needle biopsy is extensively performed by clinicians and radiologists in Songklanagarind Hospital. This method of diagnosis for hepatic malignancy has a sensitivity of 90%-96% and a specificity of 90%-100% (Pisharodi et al., 1995, Dsenbery et al., 1995). The percentage of histologic verification of liver cancer in the hospital is as high as 55%. Determination of histologic subtype is not a routine pathological practice in Songklanagarind Hospital because nearly all biopsy specimens are needle biopsies and the autopsy rate is very low. This is also true in other places where most of the patients are in late stages of disease and surgical resection has no better outcome than chemotherapeutic protocols. Since most of HCC's are monomorphic, it is conceivable that the histologic subtype can be determined with some degree of certainty from adequate needle biopsy specimens.

In one study, the five-year survival rate of fibrolamellar carcinoma patients who had complete tumor resection was 45 percent, with a median survival time of 50 months; the five-year survival rate of patients with hepatocellular carcinoma who had complete tumor resection was 0 percent, with a median survival time of 22 months (Wood et al., 1988). Overall median survival time of patients in Songkhla is 2.1 months (Sithinamsuwan et al., 2000). Most of the cases present at an advanced stage and are related to HBV infection.

Many series in the past have reported no association of histological subtypes and prognosis, although a few have correlated the subtypes, histologic features and clinical characteristics. A study from the US reported a poorer prognosis with the trabecular pattern than other subtypes (Chedid et al., 1999). None have ever been done in the Thai population where HBV is the main etiologic agent of HCC. This study was designed to determine the histological subtypes of hepatocellular carcinoma and their association with histologic findings and some clinical characteristics. Results of liver function tests for levels of alpha fetoprotein (AFP) were also assessed.

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## Materials and Methods

Archived microscopic slides of adult HCC- ICD-O 2nd edition (Percy et al., 1990) T22.0 M8170/3 and M8171/3 diagnosed in the Department of Pathology, Songklanagarind Hospital from 1991 to 1998 were reviewed. Seven childhood HCC cases were excluded. A total of 262 cases were found and 180 cases (68.7%) were of good enough quality and had an adequate amount of tissue to be reevaluated.

All slides were reviewed and the tumours were classified by one of the authors (HS). The WHO classification of histologic types was used as a reference (Hirohashi et al., 2000). Various microscopic details such as maximum mitoses per high power field, degree of pleomorphism, size and number of nucleoli, and degree of fibrosis, were graded by three categories - no, moderate, and marked. The tumours were graded according to the WHO grading convention (Hirohashi et al., 2000). Special features such as the presence of clear cells and hyaline globules were also noted as absent or present. When a tumour could not be classified for any reason, i.e. too few tumor cells in the slides, other/ undetermined was given as type.

Demographic data were based on those collected in the cancer registry. Clinical and laboratory data included the level of alpha fetoprotein (AFP), hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (HCV), liver function tests, signs and symptoms related to liver diseases, based on the records of Sithinamsuwan P. (Sithinamsuwan et al., 2000).

Percentage and 95% confidence interval of each finding were calculated using Stata version 7.0 (Stata Corp.). Fisher' s exact p-values were used to compare the differences of histologic characteristics by various subtypes of HCC. The relationship between AFP and other parameters was determined by the odds ratio of having AFP level greater than 400 nanogram/mL in a multiple logistic model.

## Results

Characteristics of the cases are shown in Table 1. Of all 180 cases, 147 were males and 33 were females. Age, type of biopsy specimen, religion, and follow up time did not associate with sex. Hepatitis B surface antigen was tested in 129 cases and 95 (73.6%) were positive. Hepatitis C virus was determined in only 61 cases and 4 (6.6%) were positive. No co-infection of the two viruses was found.

Table 2 shows distribution of various histologic types of HCC. Trabecular was the most common (63.3%; 95%CI: 55.8%-70.4%). Only one case of fibrolamellar hepatocellular carcinoma (ICD-O morphology code 8071/3) was found. There was no difference in distribution of histologic types by sex (p-value 0.637). Both HBV and HCV status did not correlate with histologic subtype of HCC.

**Table 1. Characteristics of 180 Hepatocellular Carcinoma Cases.**

| Characters | Male (147) | Female (33) | p-value |
|---|---|---|---|
| Age (years) | | | 0.358 [a] |
| Mean ± SD | 53.5 ± 12.9 | 55.8 ± 14.3 | |
| Range | 21-79 | 20-81 | |
| Specimen | | | 0.900 [b] |
| Needle biopsy | 128 | 30 | |
| Open biopsy | 13 | 2 | |
| Tumor resection | 6 | 1 | |
| Religion (164 cases) | | | 0.152 [b] |
| Buddhist | 124 | 28 | |
| Christian | 0 | 1 | |
| Islam | 10 | 1 | |
| HbsAg | | | 0.089 [c] |
| positive | 82 | 13 | |
| negative | 25 | 9 | |
| not done | 40 | 11 | |
| HCV antibody | | | 0.240 [c] |
| positive | 3 | 1 | |
| negative | 44 | 13 | |
| not done | 100 | 19 | |
| Follow up time (days) | | | 0.589 [a] |
| Median | 61.5 | 72 | |
| Range | 0-1914 | 4-671 | |

[a]t-test, [b]Fisher's exact test, [c]chi-squared test for positive and negative results

Histologic characteristics of the three major types, trabecular/pseudoglandular, compact, and scirrhous, are listed in Table 3. There was no difference among the major types in any aspect except that bile pigment was marginally more common in scirrhous and compact than trabecular/ pseudoglandular types. Parameters not listed in the Table including pleomorphism of the nucleus, presence and number of the nucleoli, intranuclear inclusion, clear cell appearance, necrosis, fibrosis, and small cell size were not associated with subtypes. Malorie bodies were present in only 2 cases, pale bodies in 3 cases, ground glass cytoplasm in 4 cases and fat vacuoles in 8 cases.

Table 4 shows the correlation between alpha fetoprotein

**Table 2. Types of Hepatocellular Carcinoma (ICD-O C22.0 8070/3 and 8071/3)**

| Type | Male (147) | Female (33) | Total (180) | Percentage | 95% CI |
|---|---|---|---|---|---|
| Trabecular | 94 | 20 | 114 | 63.3 | 55.8 - 70.4 |
| Pseudoglandular | 7 | 2 | 9 | 5.0 | 2.3 - 9.3 |
| Compact | 25 | 3 | 28 | 15.6 | 10.6 - 21.7 |
| Scirrhous | 10 | 4 | 14 | 7.8 | 4.3 - 12.7 |
| Fibrolamellar | 1 | 0 | 1 | 0.6 | 0.0 - 3.1 |
| Other/Undetermined | 10 | 4 | 14 | 7.8 | 4.3 - 12.7 |

**Table 3. Histologic Characteristics of Various Types of 165 Hepatocellular Carcinomas**

| Characters | Trabecular | Compact | Scirrhous | p-value [a] |
|---|---|---|---|---|
| Mitosis | | | | 0.108 |
| Infrequent | 97 | 18 | 13 | |
| Frequent | 26 | 10 | 1 | |
| Apoptosis | | | | 0.169 |
| Infrequent | 106 | 20 | 12 | |
| Frequent | 17 | 8 | 2 | |
| Hyaline globule | | | | 0.169 |
| Absent | 113 | 27 | 11 | |
| Present | 10 | 1 | 3 | |
| Bile | | | | 0.054 |
| Absent | 43 | 6 | 1 | |
| Present | 80 | 22 | 13 | |
| WHO grade | | | | 0.181 |
| well differentiated | 10 | 0 | 0 | |
| moderately differentiated | 76 | 4 | 8 | |
| Poorly differentiated | 37 | 14 | 6 | |

Trabecular = trabecular and pseudoglandular types. Fibrolamellar, other and undertermined types are excluded. [a] Fisher's exact test.

and some histologic and laboratory parameters. Tumours with clear cell features tended to have a higher level of AFP, however, the p-value and the likelihood ratio tests showed a non-significant association. In contrast, cases with hyaline globules in the tumour cells had lower levels of AFP. Though this association is not significant by the p-value, the likelihood ratio test gives a significant change if the parameter is omitted from the model. AST/ALT ratio is stratified into 3 levels:- less than 2, 2 to 4, and greater than 4. Figure 1 shows an increase in AFP levels when the AST/ALT ratio increases. The logistic model also shows the same trend, as presented in Table 4.

## Discussion

The major risk factor of hepatocellular carcinoma in Thailand is the hepatitis B virus (OR 15.2; 95%CI: 2.3-102.8). In our study, the prevalence of HBsAg among HCC cases of 73.6% is higher than that in the study of Srivatanakul et al. (1991a) done in northeastern Thailand which was 36.9%. The prevalence of HBV in HCC is around 70% in Asian populations (Pawarode et al., 2000, Merican et al., 2000). Among Japanese, the percentage of HBV in HCC cases is 33%. The prevalence of HBV infection marked by the presence of antibodies or antigens among Songkhla people surveyed in late 1980 decade (Srivatanakul et al.,1991b) was 41.5% and the prevalence of HBsAg positive was reported at 4.0% to 10% (Srivatanakul et al., 1991b; Merican et al., 2000). The prevalence of HBsAg in the Thai population is not different from that in other Asian populations. The prevalence of HBV and HCV among patients with HCC in European populations was 9.8-10% for HBV and 38-85% for HCV depending on the country (CLIP, 2000; Schoniger-Hekele et al., 2001). The prevalence of hepatitis C virus of 6.6% in our study was similar to that among cases and also controls in the study of Srivatanakul et al. (1991a) and was slightly lower than that in European populations. It was much lower than that in Japan which was 63% (Ikeda et al., 1993).

There are some other risk factors reported in Thailand. Betel quid chewing is also a probable risk factor of HCC (OR 11.0; 95%CI: 1.0-115.8) while alcohol consumption is a weak risk factor (3.4; 95%CI: 0.8-14.6; Srivatanakul et

**Table 4. Correlation between alpha fetoprotein and histologic and laboratory parameters.**

| Factors | Odds ratio | 95% CI | P-value |
|---|---|---|---|
| Clear cell | 1.95 | 0.90 – 4.24 | 0.091 |
| Hyaline globule | 0.14 | 0.02 – 1.29 | 0.083 |
| AST/ALT ratio* | 1.76 | 1.03 – 2.99 | 0.038 |

* AST/ALT ratio is categorized into 3 levels:- less than 2, 2 to 4, and greater than 4.

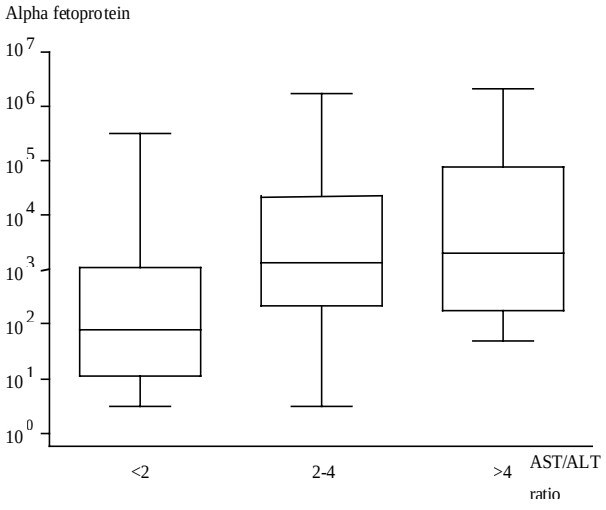


**Figure 1. Alpha Fetoprotein in Patients with Three Categories of AST/ALT Ratio**

**Table 5 Types of hepatocellular carcinoma classified by cirrhosis of patients.**

| Subtype | Sooklim (2003) | | | | Bralet (2000) | Chedid (1999) | Nzeako (1996) | | Kemeny (1989) | Nakashima (1983) | Okuda (1980)* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | C (%) | NC (%) | Unk (%) | Com (%) | NC (%) | Com(%) | C(%) | NC(%) | C (%) | Com (%) | Com % |
| Trabecular | 54(68.4) | 40(63.4) | 20(52.6) | 114(63.3) | 47(62.7) | 97(43.5) | 301(65.2) | 175(51.2) | 21(80.7) | 164(71.3) | (75.5) |
| Pseudoglandular | 7(8.8) | 1(1.6) | 1(2.6) | 9(5.0) | 16(21.3) | 28(12.5) | 25(5.4) | 12(3.5) | 3(11.5) | 27(11.7) | (9.3) |
| Compact | 9(11.4) | 9(14.3) | 10(26.3) | 28(15.6) | 4(5.3) | 22(9.9) | 54(11.7) | 81(23.7) | 1(3.9) | 16(7.0) | (13.9) |
| Scirrhous | 2(2.5) | 11(17.5) | 1(2.6) | 14(7.8) | 0 | 13(5.8) | 2(0.4) | 1(0.3) | 0 | 10(4.3) | (1.1) |
| Fibrolamellar | 1(1.3) | 0 | 0 | 1(0.6) | 8(10.7) | 4(1.8) | 7(1.5) | 37(10.8) | 0 | 0 | |
| Other/Undetermined | 6(7.6) | 2(3.2) | 6(15.8) | 14(7.7) | 0 | 59(26.5) | 73(15.8) | 36(10.5) | 1(3.9) | 13(5.7) | (0.2) |
| Total | 79(100) | 63(100) | 38(100) | 180(100) | 75(100) | 223(100) | 462(100) | 342(100) | 26(100) | 230(100) | 2411 |

* Percent from 2411 cases
C = Cirrhosis, NC = Non-cirrhosis, Unk = Unknown, Com = Combined cirrhotic and non-cirrhotic

al., 1991a). Aflatoxin seems to have no important role in hepatocarcinogenesis in Thailand (Hollstein et al., 1993).

The problem of misclassification might have occurred in this study even though we did not attempt to classify the tumours of which the tissue was too small to be evaluated. The amount of tissue from a needle biopsy might not represent the whole tumour, and tissue distortion may lead to over-estimation of a compact subtype. The focal aggregation of collagenous stroma present in needle biopsy specimens may also lead to over-interpretation of the scirrhous subtype.

The trabecular and pseudoglandular subtypes in our study accounted for 68.3% of all tumours, which is similar to that reported in other studies, which varied from 70-80% (Bralet et al., 2000; Kemeny et al., 1989; Nakashima et al., 1983; Okuda, 1980). However, a tendency of a higher proportion of scirrhous types in Asian populations than in western countries is also observed, as presented in Table 5 (Bralet et al., 2000; Chedid et al., 1999; Nzeako et al., 1996 Nakashima et al., 1983; Kemeny et al., 1989; Okuda et al., 1980). Controlled for reported series, recalculation of the data in Table 5 shows no correlation between cirrhosis status and proportion of subtypes. Though Nzeako reported a high proportion of fibrolamellar carcinomas among non-cirrhotic patients, others reported no difference in proportion of this subtype by cirrhosis status. There was no correlation between histologic characteristics of tumour cells and histologic subtypes as shown in Table 3.

The presence of clear cells and a high AST/ALT ratio correlates with a high AFP level while hyaline globules tend to be present in those with low AFP values (Table 4). A clear cell appearance has been shown to be positively correlated with AFP levels (Panchanadam et al., 1980). It has been mentioned that hyaline globules are possibly AFP stored in carcinoma cells (Cohen, 1976), but a correlation with serum AFP levels was not hypothesized. Our study suggests low AFP levels among those who have hyaline globules in their carcinoma cells, suggesting a complex role of AFP release from carcinoma cells into the blood stream and hyaline globule formation. This study finds a positive correlation of AFP level and AST/ALT ratio as previously reported by Nomura et al. (1989). Such an association may be due to the release of AFP together with AST from carcinoma cells in the process of tumour growth, necrosis, or progression.

In conclusion, the histologic subtypes of HCC in the southern Thai population are trabecular 63.3%, compact 15.6%, scirrhous 7.8%, pseudoglandular 5%, and fibrolamellar 0.6%. The difference between our findings and other series in which HCV is the main etiologic factor for HCC suggests that whenever HCC is induced by a virus it shares a common natural history. There is no correlation of histologic subtypes with hepatitis virus status or with histologic findings and cirrhotic status. A correlation between AFP levels and AST/ALT ratio was also found.

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