

Histopathological landmarks of hepatocellular carcinoma in Malaysians

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Abstract

A study was conducted at the Department of Pathology, University of Malaya Medical Centre, Kuala Lumpur into the histological type (WHO classification), grade (modified Edmondson and Steiner's grading system), mitotic rate, bile production, hyaline globule and Mallory hyaline formation of 52 cases of hepatocellular carcinoma (HCC) diagnosed during a 13-year period between 1st January 1990 to 31st December 2002. In addition, associated cirrhosis, dysplasia (large liver cell dysplasia: LLCD and small liver cell dysplasia: SLCD) and microvascular permeation were also looked for whenever the situation permitted. The patients' ages ranged from 21-years to 85-years (mean = 58.7 years) with a predilection for males and Chinese. Histologically, majority (73.1%) of the tumours demonstrated a trabecular pattern of growth. The bulk (73%) of the tumours were either of grade II or III differentiation. Mitotic activity ranged between 0-100/10 high power fields (hpf) with a mean of 22.2/10 hpf. Bile was noted in 25%, hyaline globules 17.3% and Mallory bodies in one case. Concomitant cirrhosis was present in 73.5%. 73.5% of the cases had associated LLCD. 5 with LLCD also showed SLCD. Microvascular permeation was shown in 76.2% of cases. On comparison with findings from other studies, no major difference seems to exist between the histological characteristics of our HCC cases and that of other populations.

Key words: Hepatocellular carcinoma, Malaysians, histological parameters

INTRODUCTION

Hepatocellular carcinoma (HCC) is the eighth most common cancer in the world, and is especially important in the high risk regions of East and South-East Asia and sub-Saharan Africa.¹ In Malaysia, HCC is an important entity being the 2nd most common malignancy resulting in deaths in 1995.² Although majority of HCC require histological confirmation of the diagnosis prior to institution of definitive treatment, there has been no study to date to document the histological characteristics of this tumour in the Malaysian setting. As the pattern of HCC is known to differ between geographic locations, it was felt that it would be important to establish a baseline of diagnostic histological parameters in our population. In the process, the results will also serve to verify whether substantial differences exist between histopathological features of HCC in Malaysians compared with other populations.

Histological type, grade, mitotic rate, bile production, evidence of hyaline globules and Mallory bodies in the tumour as well as presence of concomitant cirrhosis, dysplasia and microvascular permeation were analysed in cases of HCC diagnosed at the Department of Pathology, University of Malaya Medical Centre, Kuala Lumpur (UMMC) during the 13-year period between 1st January 1990 till 31st December 2002.

MATERIALS AND METHODS

All cases of HCC diagnosed at the Department of Pathology, UMMC from 1st January 1990 till 31st December 2002 were retrieved from the department files. The demographic characteristics of the cases were obtained from the histopathology request forms. The haematoxylin and eosin (H&E) stained slides of all cases were retrieved and reviewed. Only histologically re-confirmed cases were admitted into the study.

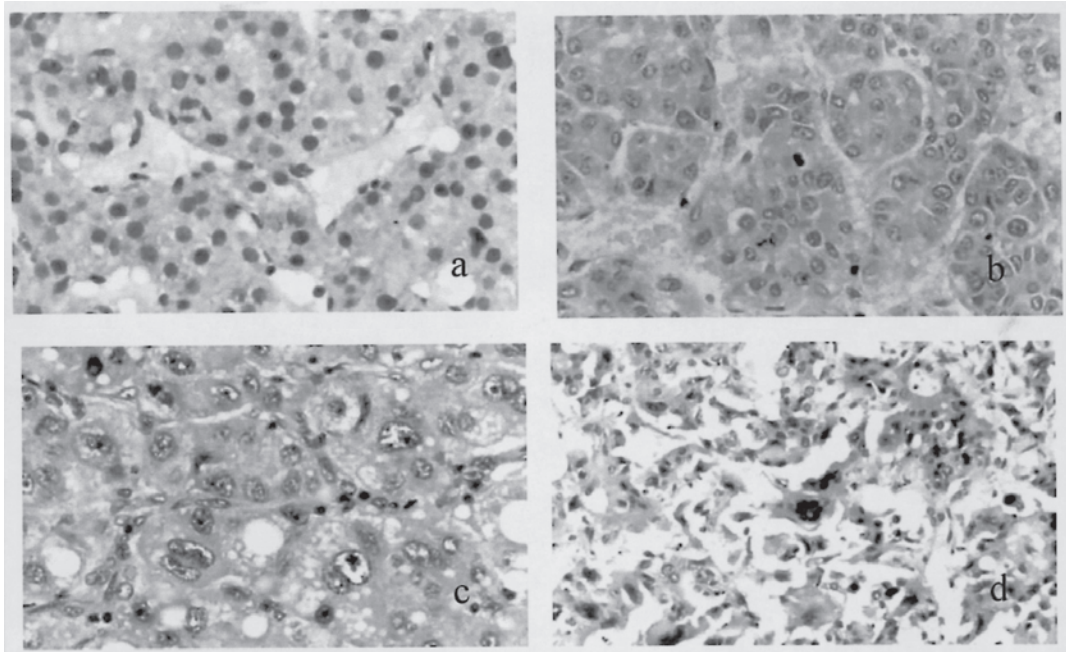


FIG 1: Examples of hepatocellular carcinoma graded according to a modified Edmondson and Steiner nuclear grading system. (a) Grade I tumour with nuclei showing minimal irregularity, (b) Grade II with increased nuclear irregularity and appearance of nucleoli, (c) Grade III with more marked nuclear pleomorphism and (d) Grade IV with anaplastic features.

The histological typing of the tumours was based on the WHO classification system.³ Whenever more than one histological pattern was seen, the tumour was classified according to the predominant pattern i.e. the pattern seen in >75% of the tumour. A mixed pattern was only considered if there was no predominant pattern. The tumours were graded using a modified Edmondson and Steiner's grading system^{4,5} into four grades with Grade I tumours having cells with abundant cytoplasm and minimal nuclear irregularity (Figure 1a). Grade II tumours exhibited greater nuclear irregularity and prominent nucleoli (Figure 1b). In Grade III tumours, there was increased nuclear pleomorphism and angulation of the nuclei (Figure 1c). Tumour giant cells were also more commonly seen. The cells in Grade IV tumours were poorly differentiated with marked nuclear pleomorphism, hyperchromatism and anaplasia (Figure 1d). All the tumours were graded according to the worst area of differentiation. Mitoses were counted in a minimum of 10 consecutive high power fields (10 x 40 magnification on an Olympus BH-2 microscope). Only unequivocal mitoses were counted and the mitotic index was rated as number of mitoses in 10 high power fields (hpf). All the H&E stained

slides of each case were searched for bile production as well as presence of hyaline globules and Mallory bodies. Whenever possible, concomitant cirrhosis, dysplasia and microvascular permeation were also looked for. Dysplasia was categorised as large liver cell dysplasia (LLCD) or small liver cell dysplasia (SLCD) according to Anthony et al⁶ and Watanbe et al's⁷ criteria respectively. LLCD was defined as groups of hepatocytes with nuclear and cellular enlargement, normal nucleocytoplasmic ratio, nuclear pleomorphism, multinucleation and prominent nucleoli (Fig. 2) while SLCD meant groups of hepatocytes with increased nucleo-

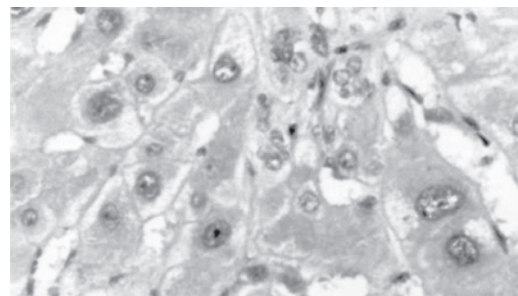


FIG 2: Large liver cell dysplasia with nuclear pleomorphism occurring in hepatocytes having normal nucleocytoplasmic ratio.

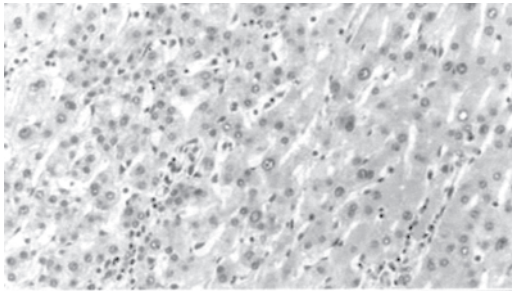


FIG 3: Case showing an area (upper left) of small liver cell dysplasia with hepatocytes exhibiting mild nuclear pleomorphism but having increased nucleo-cytoplasmic ratios giving rise to an appearance of nuclear crowding which identifies the area on low power.

cytoplasmic ratio and some nuclear pleomorphism leading to an impression of an area of nuclear crowding (Fig. 3).

Cases in which the H&E slides could not be retrieved were rejected from the study. In addition, cases where the stain of the diagnostic tissue section was faded were also not entered into the study. Whenever a case had more than one representative section of the tumour, at least one section adequately stained for microscopic examination had to be available before it could be admitted into the study.

RESULTS

In the period from 1st January 1990 till 31st December 2002, HCC was either diagnosed or suggested as the most likely histological diagnosis in 120 cases at the Department of Pathology, UMMC. Of these, the slides of 55 cases could not be retrieved. Slides of 65 cases were retrieved and reviewed histologically. On review, one case was re-classified as cholangiocarcinoma with another two as metastatic lesions (adenocarcinoma and neuroendocrine carcinoma). In 10 cases, the tumour tissue was either not sufficient or the staining too faded for confirmatory diagnosis. Finally 52 cases were entered into the study. Table 1 shows the demographic profile of the cases of HCC. Of the 52 cases, there were 44 males and 8 females with a male : female ratio of 5.5 : 1. The patients' ages ranged from 21 years to 85 years with a mean of 58.7 years. Ethnically, there were 43 Chinese, 5 Malay and 3 Indian patients with one of minority ethnic origin.

TABLE 1: Demographic profile of cases of hepatocellular carcinoma (n=52)

Sex	Male	44
	Female	8
	M : F	5.5 : 1
Age (years)	Range	21 - 85
	Mean	58.7
Race	Chinese	43 (82.7%)
	Malay	5 (9.6%)
	Indian	3 (5.8%)
	Others	1 (1.9%)

Table 2 tabulates the histological type, grade, mitotic rate, bile production, hyaline globules and Mallory bodies presence of the 52 HCC cases. The trabecular pattern was the most commonly encountered and formed 73.1% of the cases. This was followed by tubular (11.5%), clear cell (7.7%), solid (5.8%) and sclerosing (1.9%). No mixed or sarcomatoid pattern was noted. Grade II and III tumours were equally prevalent and each formed 36.5% of the cases. 17.3% of the HCC were grade IV and 9.6% grade I. Mitotic rate ranged between 0-100 per 10 hpf with a mean of 22.2/10 hpf. Bile was produced in 25.0% of the cases. Hyaline globules and Mallory bodies were noted in 17.3% and 1.9% of the cases respectively.

Cirrhosis and dysplasia could only be assessed in 34 cases. In 18, inadequate non-tumour tissue was available for assessment of the above. Associated cirrhosis was found in 25 (73.5%) and was absent in 9. Of the 9 cases without evidence of cirrhosis, assessment for cirrhosis was done in segmentally resected liver in 8 and trucut biopsied tissue in one. LLCD was present in 25 (73.5%) cases. 5 of the cases with LLCD also showed coexistent SLCD, hence SLCD was noted in 14.7% of HCC cases. In the 9 cases, where dysplastic foci of either LLCD or SLCD types were taken to be absent, 5 were studied in resected liver and 4 in trucut biopsied tissue. Microvascular permeation was noted in 76.2% of 42 cases where sufficient tissue was available for study of this parameter. Table 3 summarises the association of cirrhosis, dysplasia and microvascular permeation in HCC.

TABLE 2: Histological features (microscopic appearance, differentiation, mitotic activity, bile production, hyaline globules and Mallory hyaline bodies) in hepatocellular carcinoma (n=52)

Histological type	Trabecular	38 (73.1%)
	Tubular	6 (11.5%)
	Solid	3 (5.8%)
	Sclerosing	1 (1.9%)
	Clear cell	4 (7.7%)
	Sarcomatoid	0
Grade	I	5 (9.6%)
	II	19 (36.5%)
	III	19 (36.5%)
	IV	9 (17.3%)
Mitotic rate (per 10 hpf)	Range	0-100
	Mean	22.2
Bile production	Present	13 (25.0%)
	Absent	39 (75.0%)
Hyaline globules	Present	9 (17.3%)
	Absent	43 (82.7%)
Mallory bodies	Present	1 (1.9%)
	Absent	51 (98.1%)

TABLE 3: Associated features seen in the cases of hepatocellular carcinoma (n=52)

Cirrhosis	Present	25 (73.5%)	
	Absent*	9 (26.5%)	
	Not assessed	18	
Dysplasia	Present	Large cell	25 (73.5%)
		Small cell**	5 (14.7%)
	Absent***	9 (26.5%)	
	Not assessed	18	
Microvascular permeation	Present	32 (76.2%)	
	Absent	10 (23.8%)	
	Not assessed	10	

* 8 cases were assessed on segmentally resected liver tissue

** All cases with small liver cell dysplasia also showed coexisting foci of large liver cell dysplasia

*** 5 cases were assessed on segmentally resected liver tissue

DISCUSSION

This study was initiated with the objective of establishing a baseline of histopathological characteristics in hepatocellular carcinomas of our local population. While HCC was either histologically diagnosed or suggested as the most likely histological diagnosis in 120 cases received at the Department of Pathology, UMMC during the 13-year period between 1st January 1990 till 31st December 2002, only 55 cases were considered for the study and 52 finally admitted. While majority of the remaining 65 cases could have been considered if recuts were made from the tissue blocks, a decision was taken not to recut the tissue blocks to avoid loss of material for future study. On the same premise, no attempt was made to perform any additional histochemical or immunohistochemical stain on the tissues in a systematic manner which could have allowed for meaningful interpretation of some other important parameters e.g. hepatitis B markers, alphafetoprotein, alpha-1-antitrypsin expression etc.

With the above limitations in mind, the study showed several interesting results. The male predominance with a male:female ratio of 5.5:1 lies within the range commonly reported worldwide.⁸⁻¹⁴ The predilection of HCC for Chinese is similar to that observed in earlier studies carried out in this country and neighbouring Singapore.^{8-11, 15, 16} The mean age at presentation of the cases, 58.7 years, also did not differ from earlier studies conducted in this region.⁹⁻¹¹

Histologically, the trabecular type was the most commonly encountered variety and formed 73.1% of the tumours. This finding is similar to that noted by most other workers; the trabecular type being reported to be the most common type and constituting 60-75% of HCC.^{5, 17, 18} Nonetheless, in a Zairian study, trabecular forms were only observed in 31.4% of cases,¹⁹ giving the impression that histological patterns vary according to locale. Grade II and III tumours together formed 73% of the cases in this study. This predominance of grade II and III tumours with both totaling 75-85% of HCC has been noted by other workers.^{20, 21} Marked variation was observed in the mitotic rate with some tumours exhibiting no mitosis to those which had 100 mitoses per 10 hpf. As mitotic index is currently believed to be an important independent prognostic parameter in HCC,²²⁻²⁵ this range is not surprising as no attempt was made to select the tumours. Careful search revealed bile

production in only 25% of the cases. This seems slightly higher than the 12% in Cohen's study.²⁶ As majority of cases do not appear to demonstrate histologically detectable bile production, the presence of bile although specific for tumours of liver cell origin seems to have a low sensitivity.²⁷ Hyaline globules, intra and extracellular, were observed in 17.3% of HCC. It is interesting that the frequency of hyaline globules in HCC seems to vary widely between studies with hyaline globules being noted from 1% to 45%.²⁷⁻²⁹ Whether this difference has any association with different underlying environmental or genetic conditions needs further clarification. Mallory bodies were noted in one case, making this an unusual finding.

Thirty-four of the 52 HCC cases were deemed to have sufficient adjacent benign liver tissue for assessment of cirrhosis and dysplasia. Cirrhosis was present in the adjacent liver in 73.5% of the cases. This seems higher than 53% recently reported by Sim et al¹¹ in Singapore but prevalence rates of concurrent cirrhosis in HCC are known to differ between studies and have ranged from 50% to 100%.³⁰⁻³² LLCD was found in the adjacent liver of 73.5% of cases. In comparison, SLCD, the entity currently thought to have more premalignant potential than LLCD, was found only in 14.7% of cases.³³ Most studies have also shown that SLCD is found in lesser numbers than LLCD both in cirrhosis and HCC but figures vary with centres.³⁴⁻³⁶ SLCD presenting together with LLCD, as seen here, has also been reported.³⁴ Microvascular permeation was present in 76.2% our cases. Similar to mitotic index, microvascular permeation has also been shown to be an important prognostic marker for HCC.^{37, 38} Although it would appear that microvascular permeation occurred more frequently among our cases compared with the 30-50% observed in other studies, as for mitotic index, it has to be taken in the context that cases in this study were not selected. Those deemed inoperable and in advanced disease stage but who had trucut biopsied tissue for histological examination were also included. In comparison, the lower microvascular permeation rates reported by other workers are based on studies in explanted or surgically resected liver specimens.^{37, 38}

In summary, it appears that probably no major difference exists in the histological characteristics of HCC in our population when compared with others.

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