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Computer-linked image analysis of nuclear area: is there a use in diagnosis and grading of hepatocellular carcinoma?

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Abstract

Hepatocellular carcinoma (HCC) ranks as the fifth most common cancer with an increasing frequency worldwide. "Nuclear atypia", one of the critical features in histological diagnosis of malignancy and grading of the tumour, is generally ascertained through eyeballing. A study was conducted at the Department of Pathology, University of Malaya Medical Centre to assess whether nuclear area, (surrogate measure for nuclear size) and standard deviation (surrogate measure for nuclear pleomorphism) when objectively measured via computer-linked image analysis differs between (1) benign and malignant liver cells and (2) different grades of HCC. A 4-µm thick H&E stained section of 52 histologically re-confirmed HCC with 36 having benign, non-dysplastic surrounding liver were analysed using the Leica Q550 CW system. 10 consecutive non-overlapping, non-mitotic and non-apoptotic nuclei of HCC and surrounding benign hepatocytes respectively were manually traced at 400x magnification on the computer monitor and the nuclear area for the particular cell computed in arbitrary units by the Leica QWIN software. A total of 360 benign hepatocytic nuclei, 240 low grade HCC and 280 high grade HCC nuclei were traced. The mean nuclear area of the benign hepatocytes (37.3) was significantly smaller ($p<0.05$) than that of both low grade (65.2) and high grade HCC (80.0). In addition, the mean nuclear area of high grade HCC was significantly larger ($p<0.05$) than the low grade HCC. SD of the nuclear areas was lowest in benign hepatocytes (9.3), intermediate in low grade HCC (25.0) and highest in high grade HCC (25.6). These findings indicate that computer-linked nuclear measurement may be a useful adjunct in differentiating benign from malignant hepatocytes, in particular in small biopsies of well-differentiated tumours, and in predicting survival after surgical resection and transplant.

Key words: Hepatocellular carcinoma, computerized morphometry, nuclear area, tumour grade

INTRODUCTION

Hepatocellular carcinoma (HCC) ranks as the fifth most commonly encountered cancer and third most common cause of cancer-related mortality in the world. There is also a disturbing increase in frequency worldwide.[1, 2] For the histopathologist, one of the most confounding issues when dealing with HCC often lies in deciding whether the biopsied tissue is benign or malignant especially when the biopsy yields limited diagnostic material or when the tumour is well-differentiated. To date, effective treatment modalities for HCC are still limited to surgical resection and transplant. Careful choice of patients for radical surgery is dependent on anatomic location of the tumour but survival after surgery is often dictated by parameters which influence recurrence. Tumour grade has been shown to be one of the most significant factors affecting post-surgical recurrence and survival.[3-5] This leaves the histopathologist with the second problem of determination of degree of "nuclear atypia" and thenceforth grading the tumour. In most cases, classification of "nuclear atypia" is through eyeballing and making a subjective interpretation of how the tumour nuclei differ from what is expected in their normal counterparts. Generally, an increase in the size of the nuclei with added features of pleomorphism implies malignant transformation with more pronounced changes suggesting a higher grade of tumour. Although usually accepted as such, little work has been carried out to correlate objective measurements of nuclear parameters with malignant transformation or grading of tumours. We were therefore interested to assess whether nuclear area, (surrogate measure for nuclear size) and standard deviation (surrogate measure

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for nuclear pleomorphism) when objectively measured via computer-linked image analysis differs (1) between benign and malignant liver cells and (2) in different grades of HCC.

MATERIALS AND METHODS

Fifty-two cases of hepatocellular carcinoma, diagnosed at the Department of Pathology, University of Malaya Medical Centre between 1st January 1990 and 31st December 2002, and entered into an earlier study by the authors[6] were admitted into this study. Only cases where at least one diagnostic HCC tissue section which was adequately haematoxylin and eosin (H&E)-stained and could be retrieved from the department files were entered into the earlier study. Cases where the H&E-stain of the diagnostic tissue section was faded were not entered. Among the 52 cases of HCC, 36 had benign, but not dysplastic, surrounding liver tissue in the same diagnostic tissue section. This surrounding benign liver could be cirrhotic or non-cirrhotic. For the present study, one adequately stained 4 μm thick haematoxylin-eosin (H&E) section containing tumour of each case was selected for computer-linked image analysis using the Leica Q550 CW system. All the cases had been histologically reviewed, reconfirmed and graded according to the Edmondson and Steiner's grading system[7,8] during the earlier study. For the purposes of this study, the cases were stratified into two rather than four grades viz low grade incorporating Edmondson and Steiner's grades I and II and high, grades III and IV, in view of the small number of grade I and IV HCC cases. For HCC, an area was identified on each H&E-stained section where there were at least 10 consecutive non-overlapping, non-mitotic and non-apoptotic malignant hepatocytic nuclei and chosen for study. For the 36 with surrounding benign liver, an area with 10 consecutive non-overlapping, non-mitotic and non-apoptotic benign hepatocytic nuclei, was selected for each case respectively for study. The outlines of each of the 10 consecutive HCC (Figure 1) and benign hepatocytic nuclei were manually traced at 400x magnification on the computer monitor. The area within the outline was considered the nuclear area for the particular cell and computed in arbitrary units by the Leica QWIN software. This data, viz computed nuclear area, was exported to Microsoft Excel 2003. The mean area and standard deviation of the areas of the tumour nuclei were calculated using Microsoft Excel 2003. Statistical analysis was carried out using the student's t-test.

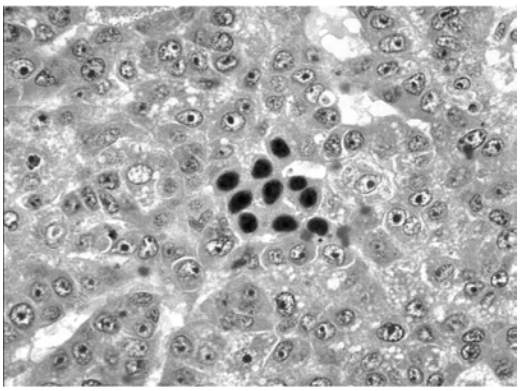

FIG. 1: The outlines of 10 consecutive HCC cellular nuclei manually traced at a 400x magnification using the Leica Q550 CW

RESULTS

Of the 52 cases of HCC entered into the study, 44 were males and 8 females with a male:female ratio of 5.5:1. Ethnically, there were 43 (82.7%) Chinese, 5 (9.6%) Malay, 3 (5.8%) Indian and 1 (1.9%) from a minority ethnic group. The patients' ages ranged from 21-years to 85-years with a mean of 58.7-years. Demographic data of the cases is shown in Table 1. 5 cases were classified as grade I, 19 grade II, 19 grade III, and 9 grade IV according to the Edmondson and Steiner's grading system and stratified as 24 low and 28 high grade HCC. A total of 360 benign hepatocytic nuclei, 240 low grade HCC and 280 high grade HCC nuclei were traced. Table 2 summarises the comparison of mean nuclear area and standard deviation (SD) of the different grades of HCC and benign hepatocytes. The mean nuclear area of the benign hepatocytes (37.3) was significantly smaller ($p<0.05$) than that of both low grade (65.2) and high grade HCC (80.0). In addition, the mean nuclear area of high grade HCC was significantly larger ($p<0.05$) than the low grade HCC. SD of the nuclear areas was lowest in benign hepatocytes (9.3), intermediate in low grade HCC (25.0) and highest in high grade HCC (25.6).

DISCUSSION

The results of this study re-affirms the general visual impression and findings of Kuo et al,[9] Koutselini et al[10] and Zeppa et al,[11] that benign had significantly smaller nuclear areas than

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%)

malignant hepatocytes. This was irrespective of grade of tumour. Furthermore, since the surrounding benign liver in the cases admitted into this study could be normal or cirrhotic, it appears that cirrhosis did not significantly alter the hepatocytic nuclear area. Zeppa at el[11] had similarly reported that well-differentiated HCC could be distinguished from cirrhosis based on the larger nuclear area of the former and their work also supports this observation.

Apart from distinguishing benign from malignant hepatocytes, mean nuclear area was significantly larger in high compared with low grade HCC and this has also been noted by Koutselini et al[10] and Ikeguchi et al.[12] Although, these findings would seem to be within general expectations, the limited number of cases did permit proper analysis of nuclear areas against the four grades of the Edmondson and Steiner's grading system. It would be interesting therefore to extend the study further to encompass more grades I and IV cases for firmer conclusions.

As nuclear pleomorphism is one of the important visual factors used in distinguishing benign from malignant cells and in the grading of malignant cells, this study also makes a noteworthy observation that the nuclear areas of benign hepatocytes demonstrated a lower SD compared with HCC cells. Furthermore, the nuclear areas of low grade HCC also exhibited a lower SD from those of high grade HCC. Thus, not only are the nuclear areas of benign smaller than malignant hepatocytes, objective measurements also showed that they had more uniform nuclear areas. Similarly, low grade HCC cells also had less variability of their nuclear areas compared with high grade.

As a projection from the findings of this study, it may be worth considering use of computer-linked nuclear measurement as an adjunct in differentiating benign from malignant hepatocytes, in particular when faced with small biopsies of well-differentiated tumours. Naturally before embarking on this, a range of normal hepatocytic nuclear areas built on a significant number of cases has to be established. As grade has been shown to be an important predictor of survival after tumour resection and transplant,[3-5] computer-linked nuclear measurement may also provide a more objective method of predicting prognosis.

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TABLE 2. Nuclear area of low grade (I+II) versus high grade (III+IV) hepatocellular carcinoma

		Nuclear Area	
		Mean	Standard Deviation
Benign liver cells (n=36)		37.3	9.3
Hepatocellular carcinoma (n=52)	Low grade (n=24)	65.2	25.0
	High grade (n=28)	80.0	25.6

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