

ORIGINAL ARTICLE

Association of Ki67 with raised transaminases in hepatocellular carcinoma

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Abstract

Transaminase enzymes, alanine (ALT) and aspartate transaminase (AST), have been reported to be raised and implicated to have prognostic value in hepatocellular carcinoma (HCC). Ki67, a marker of cellular proliferative activity, has also been noted to be increased in HCC. A study was conducted at the Department of Pathology, Faculty of Medicine, University of Malaya, Kuala Lumpur to determine the possible association of proliferative activity, as determined by Ki67, with the transaminase enzymes. 31 cases of histologically diagnosed HCC who underwent tumour resection were retrieved from departmental archives. The patients' ages ranged between 40 to 79 years with a mean of 58.3 years. There was a male preponderance with M:F = 2.9:1. Ethnic Chinese formed 83.9% of the cases. 4 μ m sections, cut from the formalin-fixed, paraffin-embedded tumour tissue block of each case, were immunohistochemically stained with Ki67 (DAKO monoclonal MIB-1) using the commercial DakoCytomation EnVision+System-HRP kit. The latest ALT and AST levels, assayed within 7 days prior to tumour resection, were retrieved from the patients' case records. 24 (77.4%) HCC demonstrated elevation of either ALT and/or AST. 27 (87.1%) HCC were immunopositive for Ki67. Ki67 immunoexpression was significantly correlated with raised transaminases ($p < 0.05$). Hypothetically, the mechanism by which this phenomenon may occur may simply be release of transaminases due to destruction of hepatocytes by the cancer. Thus rising levels of the transaminases could signal a more rapid growth of the tumour and these routinely performed tests can be of prognostic value in management of HCC patients.

Keywords: Ki-67, hepatocellular carcinoma, transaminases

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most important cancer in the liver¹ and an important cancer in Malaysians, being among the ten most common cancers in the male population.² Ki67 antibody, originally raised against a crude nuclear fraction of the Hodgkin's lymphoma cell line L428, and deriving its name from "Kiel" and the 67th well in which the reaction occurred, is a marker of cell proliferation. It is a large 395 kDa nucleolar protein encoded by a single gene on chromosome 10 and expressed throughout the cell cycle except in G₀.³⁻⁷ Although HCC is a relatively important cancer, there has been a dearth of studies carried out on Ki67 and transaminases in HCC. To date, there has been hardly any reported study which correlates expression of Ki67 with

levels of transaminases in HCC. Among the few studies on Ki67 expression in HCC, some have shown a significantly higher Ki67 expression in HCC compared with benign conditions including cirrhosis and benign tumours.^{8,9} Studies have also implied that Ki67 expression has prognostic significance in HCC.^{9,10} Alanine transaminase (ALT) and aspartate transaminase (AST), also known as serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) respectively are part of the gamut of routinely performed "liver function tests" and have been noted to be increased in HCC.¹¹⁻¹⁴ Furthermore some studies have shown association of transaminases with survival of HCC patients.¹²⁻¹⁴ It is thus of interest to assess whether Ki67 expression, which provides an indication of tumour proliferative activity, is

associated with a rise of transaminases, a set of routinely performed serological assays, in HCC.

MATERIALS AND METHODS

All cases of hepatocellular carcinoma histologically diagnosed for the first time between January 1992 and December 2003 and subsequently resected at the University of Malaya Medical Centre were retrieved from the files of the Department of Pathology, Faculty of Medicine, University of Malaya. All cases where the haematoxylin and eosin stained tissue sections of the resected tumour could be retrieved for histological re-confirmation were considered for entry into the study. Cases where the haematoxylin and eosin stained sections of the resected tumour could not be retrieved were dropped from the study. Needle biopsied specimens of the cases, if available and even though carrying the same histological diagnosis, were not used for the study. Demographic data of the patients were obtained from the histopathological examination request forms submitted to the Department of Pathology with the resected specimens. The latest ALT and AST levels assayed within 7 days prior to surgical tumour resection were retrieved from the patients' case records for each case. The levels were classified as either within the normal range (ALT = 30-65 IU/L; ASST = 15-37 IU/L) or raised. Only cases which were histologically re-confirmed, had sufficient residual tumour tissue in the paraffin block for Ki67 immunohistochemical staining and had ALT and/or AST levels assayed within 7 days prior to the surgical resection were admitted. The paraffin block with tumour tissue for immunohistochemical staining was selected during the histological review. 4 µm sections were cut from the formalin-fixed, paraffin-embedded tumour tissue block of each case on to aminopropyltriethoxysilane coated slides. Antigen retrieval was carried out via microwave treatment (Energy Beam Sciences, Inc., 600 watts, 100% power) at 100°C for 20 min before immunohistochemical staining with Ki67 (DAKO monoclonal MIB-1) using the commercially available DakoCytomation EnVision+System-HRP kit. Positive controls consisting of tissue sections from a tonsil with reactive lymphoid hyperplasia and negative controls made up of substituting phosphate buffered saline for primary antibody in the

staining of the positive controls were included in each batch stained.

Based on previous studies which showed that Ki67 expression in HCC tumour cells ranged between 5-50%,^{15,16} we arbitrarily chose a cut-off value of 5%. Ki67 expression in the tumour was semi-quantitated by eyeballing and categorized as immunopositive only when >5% of tumour cells demonstrated unequivocal nuclear expression. No attempt was made to semi-quantitate the intensity of Ki67 staining. Statistical analysis was carried out using Fisher's exact test.

RESULTS

Thirty-one cases of histologically-reconfirmed hepatocellular carcinoma who had ALT and/or AST measured within 7 days prior to surgical resection of the tumour were finally admitted into the study. Of these, there were 23 males and 8 females with an M: F ratio of 2.9: 1. 26(83.9%) of the cases were Chinese, 4(12.9%) Malay and 1(3.2%) Indian. The ages of the patients ranged between 40 to 79 years with a mean of 58.3 years. Table 1 illustrates the demographic profile of the cases of HCC in the study.

Table 2 shows ALT and AST levels correlated with Ki67 expression in the hepatocellular carcinoma cases. 24 (77.4%) HCC demonstrated elevation of either ALT and/or AST. 27 (87.1%) HCC demonstrated Ki67 expression in more than 5% of tumour nuclei and were considered immunopositive for Ki67 expression (Fig 1). Among the 4 cases which did not express Ki67, only 1 (25%) case had raised transaminases. In contrast, 23 (85%) HCC which were Ki67 immunopositive had raised transaminases. Thus, Ki67 immunoexpression was significantly correlated with raised transaminases (p<0.05) in HCC.

TABLE 1: Demographic profile of the cases of hepatocellular carcinoma (n=31)

Sex distribution	M	23
	F	8
	M:F	2.9:1
Ethnic distribution	Chinese	26 (83.9%)
	Malay	4 (12.9%)
	Indian	1 (3.2%)
Age distribution	Range	40-79 years
	Mean	58.3 years

TABLE 2: Correlation of AST and ALT levels with Ki67 immunopositivity in HCC (n=31)

	Normal ALT and AST	Raised ALT and/or AST	Total
Ki67 positive	4	23	27
Ki67 negative	3	1	4
Total	7	24	31

DISCUSSION

Hepatocellular carcinoma is an important cancer among Malaysian males and the findings of this study reiterates the situation noted in the third report of the National Cancer Registry of Malaysia.² As in a previous study conducted by the authors,¹⁷ males were also affected more commonly than females in this study with a M:F ratio of 2.9: 1. There was also a similar distinct predilection for Chinese, who formed 84% of the patients in this study.

The transaminases (either ALT and/or AST) were raised in 77.4% of patients with HCC. This is slightly lower than that observed by Lopez *et al.*¹¹ who noted raised AST in 90% of hepatocellular carcinomas. Ki67 was immunopositive in 87.1% of HCC. This rate is also similar to that reported by other workers.¹⁸ What is perhaps more interesting is that Ki67 immunoeexpression was significantly correlated with raised transaminases ($p < 0.05$) in HCC. Cases of HCC with negligible proliferative activity showed a significant lack of rise in transaminases in contrast with cases where proliferative activity was recorded. This finding has not been reported before although Farinati *et al* had earlier reported that the number of Ki67

positive cells correlated directly with ALT levels in chronic liver diseases.¹⁹

It is noteworthy that no attempt was made to separate the transaminases in this study although the general dogma has been that AST is associated more with tumour growth and ALT with necrosis of hepatocytes.²⁰⁻²³ For the purposes of this study, it was postulated that both ALT and AST could be associated with proliferation of the HCC tumour cells. This assumption is made after taking cognition that to date, the roles of the AST and ALT in HCC are not totally clear. Both seem to have recorded elevations in HCC although the underlying triggers may be different for ALT and AST. The association of raised AST with HCC have been reported by several workers.²⁰⁻²² ALT, purportedly a surrogate marker of inflammatory hepatocyte necrosis, has been propagated as a predictor for malignant transformation to HCC in particular following hepatitis C infection or recurrence of hepatitis C-associated HCC following treatment.^{13,23,24} As for hepatitis B-associated HCC, the information regarding the value of ALT remains unclarified. While Japanese workers were unable to demonstrate differences in ALT levels of patients who

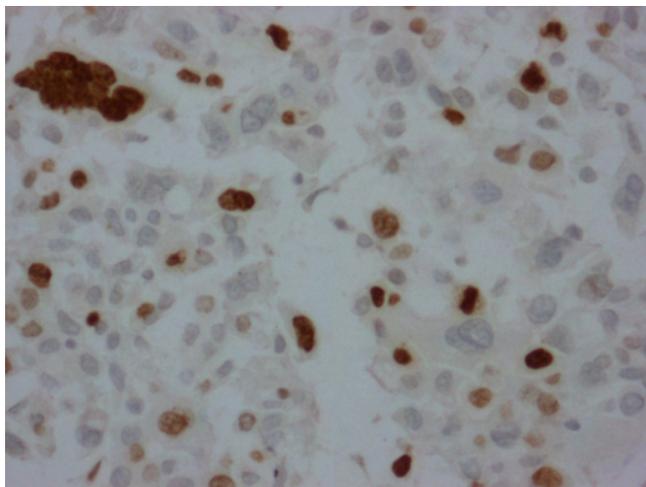


FIG. 1: A case of hepatocellular carcinoma showing Ki67 intranuclear immunoreactivity (brown) on routine immunoperoxidase staining

developed and those who did not develop HCC following hepatitis B infection,²⁵ Cheung *et al*¹⁴ noted that increased ALT levels in patients with HCC resulting from hepatitis B infection carried a higher risk of recurrence after surgical treatment.

It is well-known that serum levels of transaminases can be raised in a wide range of pathological conditions. As we have not excluded other causes of raised transaminases in this group of HCC patients, it is possible that the observed positive trend against Ki67 positivity is purely fortuitous. Nevertheless, the possibility that the rise in the transaminases in HCC is truly correlated with proliferative activity of neoplastic hepatocytes cannot be dispelled. Hypothetically, the mechanism by which this may occur may simply be the release of the transaminases resulting from destruction of hepatocytes by the cancer. Thus rising levels of the transaminases could signal a more rapid growth of the tumour and these routinely performed tests can be of prognostic value in management of HCC patients.

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