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Relationship between folate status and tumour progression in patients with hepatocellular carcinoma

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Previous studies with folate/methyl-deficient rat models proposed the role of folate deficiency in hepatocarcinogenesis and tumour progression. We investigated the relationship between folate status and tumour progression in patients with hepatocellular carcinoma (HCC). Ninety HCC patients (age 62 (SD 10) years) recruited through the Department of Internal Medicine, Chi-Mei Hospital, participated in this cross-sectional study. According to the clinical criteria, 44 % showed marginal folate deficiency (serum folate 7–14 nmol/l; folate intake 278 (SD 212) μg/d), and 16 % were folate deficient (<7 nmol/l; 207 (SD 113) μg/d). Serum folate showed inverse correlations with three elements of tumour progression: tumour size (r −0·29; P=0·005), tumour multiplicity (r −0·24; P=0·018) and metastasis (r −0·39; P=0·0001). When HCC progression was categorised into stages I to IV, serum folate decreased as HCC stage progressed (stage I, 24·5 (SD 11·5); stage IV, 10·3 (SD 3·3) nmol/l; P=0·032). After adjustment for age, sex, lifestyle and dietary factors, patients with low blood folate status (serum folate < 14 nmol/l) had increased risks for advanced tumour progression in large tumours (OR 7·1 (95 % CI 2·27, 21·9); P=0·0007), tumour multiplicity (OR 3·2 (95 % CI 1·07, 3·51); P=0·004) and metastasis (OR 4·5 (95 % CI 1·11, 18·4); P=0·03) relative to those with normal folate status. Further controlling for liver injury, tumour proliferation and tumour stage, however, negated the effect of folate on advanced tumour progression. The data thus suggest that low blood folate status could be a risk factor for tumour progression, which is modulated by clinical lesions present in HCC patients. Future studies with larger sample sizes are warranted to explore the joint effects of low folate and hepatic lesions in human HCC malignancy.

Folate status: Hepatocellular carcinoma: Tumour progression

Hepatocellular carcinoma (HCC) is the third most frequent cause of death due to malignancies in men, and its incidence is increasing worldwide[1–3]. High incidences, easy metastasis and frequent recurrence even after ablation mean that patients with advanced HCC have little chance of survival. Infection with hepatitis B and C viruses and ingestion of aflatoxin B_1-contaminated food remain the major risk factors for HCC in Asia[4]. Age, sex and alcohol-related cirrhosis are associated with HCC development[4,5]. Relatively little is known about the nutritional status of HCC patients and its relationship with HCC development. A recent prospective cohort study showed an association of low blood folate levels with risks for liver damage and HCC[6], suggesting a possible role of folate in the carcinogenesis of human HCC.

Folate plays a central role in one-carbon metabolism in the liver. An adequate folate status supplies the liver with available one-carbon carriers for *de novo* thymidylate and purine synthesis, amino acid interconversion and methylation of macromolecules[7]. Chronic liver diseases such as viral hepatitis[8,9], alcoholic liver disorder[10] and liver cirrhosis[11–13] may compromise the folate status, as low blood folate levels are commonly found in patients with those diseases. In animal studies, folate deficiency leads to genomic instability, including increased DNA strand breaks[14], hypomethylation within the *p53* tumour suppressor gene[14–16] and altered expression of genes involved in cell-cycle regulation[17], all of which have been proposed as plausible mechanisms that link folate deficiency to liver tumour progression[18]. Disturbance of one-carbon metabolism by a methyl-deficient diet (deficient in folate, choline and methionine) has been shown to promote tumour progression in rat liver[19,20]. The folate status of HCC patients in relation to tumour progression, however, has not been assessed.

Accordingly, the aims of the present study were to investigate the folate status of HCC patients and its relationship to tumour progression. Ninety HCC patients recruited through the Department of Internal Medicine, Chi-Mei Hospital, participated in this cross-sectional study. We studied serum

Abbreviations: ALT, alanine aminotransferase; GOT, glutamic-oxaloacetic transaminase; HCC, hepatocellular carcinoma; tHcy, total homocysteine.

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folate levels, dietary folate intake, general nutritional status and clinical data, including liver damage and tumour progress.

Materials and methods

Subjects

Between April 2005 and October 2006, 120 potentially eligible patients with HCC were recruited through the Department of Internal Medicine of Chi-Mei Hospital (Tainan, Taiwan). Chi-Mei Hospital provides medical services to a defined population base in southern Taiwan. Patients were diagnosed with HCC by imaging examinations, including B-type ultrasonography, computed tomography, MRI and angiography. The diagnosis of HCC progression (size, number and metastasis) was made by two physicians specialised in hepatology and oncology. For patients with a tumour size of 1–2 cm, the presence of HCC was histologically confirmed. All studied patients had primary HCC. Diagnosis of liver cirrhosis was also histologically proven. HCC patients with cardiac or renal diseases, overt diabetes, or active intravenous drug abuse were excluded. Patients with severe illness or who were unwilling to donate extra blood samples withdrew from the study. In total, ninety HCC patients participated in the entire study. Informed consent was secured from all study participants. The protocol was approved by the Committee on Medical Research of Chi-Mei Hospital and the Ethical Review Board of Fu-Jen University.

Risk factors and dietary assessment

Within 1 week following the diagnosis of HCC and before treatment in scheduled consultations, patients were asked to donate fasting blood samples and to complete questionnaires concerning their medical history, personal habits and use of medications. Demographic data, smoking status and alcohol consumption were recorded. Experienced dietitians helped patients complete a semi-quantitative FFQ covering the previous year to assess their habitual dietary folate intake. Their current folate intake was assessed by a 24 h recall at the time of the HCC diagnosis. The questionnaire was developed in our laboratory for assessing the folate intake of Taiwanese[21]. It has been validated by multiple 24 h recalls (r 0·86; $P<0·001$)[21] and by plasma folate levels (r 0·57; $P<0·001$)[8].

Blood biochemical determinations

Peripheral blood samples were taken after a 12 h fasting period, chilled, and transported to the laboratory, where serum samples were immediately separated upon arrival. The time between blood sampling and separation of serum for total homocysteine (tHcy) determinations was restricted to 2 h. tHcy levels were measured by fluorescence polarisation immunoassay (Becton Dickinson, Orangeburg, NY, USA). Serum folate was determined with a commercial RIA kit (Becton Dickinson). Serum glutamic-oxaloacetic transaminase (GOT), alanine aminotransferase (ALT) and albumin concentrations were measured by standard techniques (ITC Diagnostics, Taiwan). Hb was measured in a Coulter STKS counter (Beckman Coulter, Miami, FL, USA).

Classification of tumour progression

HCC progression was classified according to recognised criteria by the tumour, regional lymph node, and metastases (TNM) system[22]. The classification considers the presence or absence of vascular invasion, the number of tumour nodules (single *v.* multiple) and the size of the largest tumour (<5 *v.* ≥ 5 cm). In brief, primary tumour progression was categorised as T1 (solitary tumour without vascular invasion), T2 (solitary tumour with vascular invasion or multiple tumours none more than 5 cm), T3 (multiple tumours more than 5 cm or a tumour involving a major branch of the portal or hepatic veins) or T4 (with metastasis). Tumour stage grouping was defined as stages I–III for T1, T2 and T3, respectively, with no regional lymph node metastasis or distant metastasis. Stage IV was defined as any primary tumour with metastasis in any regional lymph node or distant metastasis.

Statistical analysis

Data are presented as mean values and standard deviations. According to the clinical criteria, serum folate status of individuals was classified as normal (serum folate >14 nmol/l or 6 ng/ml), marginally deficient (7–14 nmol/l or 3–6 ng/ml) or deficient (<7 nmol/l or 3 ng/ml)[23,24]. As serum folate levels of patients were stratified into various folate statuses, the absolute frequencies of categorical variables such as sex, viral infection and the presence of clinical complications were compared using the χ^2 test. Demographic and laboratory data of continuous variables were compared using one-way ANOVA followed by Duncan's multiple range test. Dependence between the folate status and tumour progression markers was evaluated using Pearson's correlation coefficient. Logistic regression models were used to estimate the OR and 95 % CI for large tumours (≥ 5 cm), tumour multiplicity (≥ 2), and metastasis with respect to normal folate status (serum folate ≥ 14 nmol/l) *v.* deficient folate status (serum folate <14 nmol/l). Non-normally distributed dependent variables were first log-transformed. Statistical analyses were performed using the Statistical Analysis System (SAS/STAT version 6.12; SAS Institute, Cary, NC, USA). Differences were considered to be statistically significant at $P<0·05$.

Results

Baseline and clinical characteristics of the studied hepatocellular carcinoma patients

Table 1 presents the baseline and clinical data of the HCC patients. Of the ninety patients, 80 % had a first-time diagnosis of HCC and 20 % had recurrent HCC within an interval of 6–24 months following their first surgical resection or transarterial chemoembolisation. A total of 44 % of patients were seropositive for hepatitis B surface antigen, 46 % were positive for serum anti-hepatitis C virus antibody, and the remaining 10 % were non-B, non-C. The mean age of patients was 62 (SD 10) years. No obvious disorders with cholesterol metabolism or kidney dysfunction were observed. Seventy-three percent had liver cirrhosis. Clinical complications included ascites (37 %), portal vein thrombosis (20 %) and hepatic encephalopathy (12 %).

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Table 1. Baseline and clinical data of ninety patients with hepatocellular carcinoma

(Mean values and standard deviations)

Variables	Mean	SD
Cases (*n*)		
New cases	72	
Recurrent cases	18	
Virological types (*n*)		
Hepatitis B virus	40	
Hepatitis C virus	41	
Sex (*n*)		
Male	65	
Female	25	
Age (years)	62	10
BMI (kg/m^2)	24	3·2
Albumin (g/l)	34·7	6·6
Cholesterol (μg/l)	161·3	48·1
Creatinine (μg/l)	1·09	0·49
Glutamic-oxaloacetic transaminase (U/l)		
Median	90·5	
Range	23–793	
Alanine aminotransferase (U/l)		
Median	67	
Range	15–446	
Cirrhosis		
n	66	
%	73	
Ascites		
n	33	
%	37	
Portal vein thrombosis		
n	18	
%	20	
Hepatic encephalopathy		
n	11	
%	12	

Demographic, lifestyle and nutritional variables by serum folate status

According to the clinical criteria, 44 % of HCC patients showed marginal folate deficiency (serum folate 7–14 nmol/l) and 16 % were folate deficient (<7 nmol/l) (Table 2). Patients with deficient serum folate had a significantly lower habitual folate intake (207 (SD 113) *v.* 336 (SD 190) μg/d; $P=0·032$), higher serum tHcy concentrations (14·1 (SD 6·2) *v.* 9·6 (SD 4·8) μmol/l; $P=0·001$) and higher frequencies of alcohol intake (36 *v.* 14 %; $P=0·019$) than those with normal serum folate levels. Serum folate levels were not significantly different by age, BMI, smoking habit, albumin level or Hb level.

Hepatic damage, clinical complications and tumour markers in hepatocellular carcinoma patients with various folate statuses

As shown in Table 3, neither liver injury (GOT and ALT levels) nor clinical complications (cirrhosis and ascites) were correlated with serum folate levels. Patients with folate deficiency had significantly higher α-fetal protein levels (1137 (SD 3838) *v.* 8 (SD 28) μg/l; $P=0·037$), larger tumours (10 (SD 14·3) *v.* 3·0 (SD 2·7) cm; $P=0·0003$), more tumours (3 (SD 0·6) *v.* 2·2 (SD 1·0); $P=0·042$) and higher metastasis rate (64 *v.* 11 %; $P=0·0002$) than those with normal folate levels (≥14 nmol/l). Values of the clinical parameters in patients with marginal folate deficiency lay between those of the folate-deficient and normal-folate groups. Pearson's correlation coefficient revealed inverse correlations between serum folate and tumour size (r −0·29; $P=0·005$), tumour multiplicity (r −0·24; $P=0·018$) and metastasis rate (r −0·391; $P=0·0001$).

Folate status and clinical factors in relation to hepatocellular carcinoma stages

When HCC progression was categorised into stages I to IV[22], tumour progression was associated with lower serum folate levels (stage I 24·5 (SD 11·5) *v.* stage IV 10·3 (SD 3·3) nmol/l; $P=0·032$), independent of folate intake (Table 4). Advanced tumour staging was associated with elevated α-fetal protein levels ($P=0·001$) and lower BMI ($P=0·032$). No significant differences in general nutritional status (albumin and Hb levels) and liver injury (GOT and ALT levels) between tumour stages were observed.

Odds ratios of hepatocellular carcinoma progression by blood folate status

We used a blood folate level of less than 14 nmol/l (the cut-off point for marginal and deficient folate levels) to represent low blood folate status for further analysis (Table 5). The OR of advanced tumour progression associated with low blood folate levels relative to normal serum folate status (≥14 nmol/l) was 7·1 for large tumours (95 % CI 2·27, 21·9; $P=0·007$), 3·2 for multiple tumours (95 % CI 1·07, 3·51; $P=0·04$) and 4·5 (95 % CI 1·11, 18·4; $P=0·03$) for metastasis after controlling for age, sex, lifestyle and dietary factors (model A; Table 5). Further controlling for liver injury, tumour proliferation and tumour stages, however, negated the effect of folate on advanced tumour progression (models B and C).

Discussion

According to the clinical criteria[23,24], 44 % of HCC patients showed marginal folate deficiency, and 16 % were folate deficient. Elevated tHcy concentrations in these patients confirmed a functional folate insufficiency[25]. The causes of low blood folate status in HCC patients are unknown. Several factors may contribute. Malnourishment is commonly found in some cancer patients[26], and may compromise the serum folate status. Impaired liver function is reported to accompany a low folate status and high tHcy levels, especially in patients with viral hepatitis[8,9], alcoholic liver disorder[10] and liver cirrhosis[11–13]. In the present study, the low serum folate levels were not associated with changes in BMI, levels of albumin, Hb, GOT or ALT, or liver cirrhosis (Tables 2 and 3). It is likely that low folate status was associated with general ill-health of HCC patients, yet was not detected due to low numbers of the study or a low predictive value of the nutritional marker. A recent study reported that folate and B vitamin status of HCC patients were reduced as HCC progressed[27]. Our data confirmed that serum folate decreased as HCC stage progressed (Table 4). The findings raise the possibility that low folate status could be an intrinsic

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Table 2. Demographic, lifestyle and nutritional variables by serum folate status of patients with hepatocellular carcinoma (HCC)†
(Mean values and standard deviations)

	Serum folate levels‡					
	Deficiency (<7 nmol/l)		Marginal deficiency (7–14 nmol/l)		Normal (>14 nmol/l)	
Variables	Mean	SD	Mean	SD	Mean	SD
Number of patients						
n	14		40		36	
%	16		44		40	
Age (years)	61·1	11·3	63·3	9·4	61·0	12·1
Sex						
Male	13*		32		20	
Female	1*		8		16	
BMI (kg/m^2)	22·5	2·7	24·4	3·5	24·2	2·9
Regular alcohol intake§						
n	5		15		5	
%	36*		28		14	
Smoking habit‖						
n	8		25		14	
%	57		62		38	
Current folate intake (μg/d)¶	134	98	196	163	189	102
% of DRI	33		49		47	
Habitual folate intake (μg/d)††	207*	113	278	212	336	190
% of DRI	47		63		76	
Serum tHcy levels (μmol/l)	14·1*	6·2	12·5	5·3	9·6	4·8
Albumin levels (g/l)	36·6	8·4	38·4	6·9	33·8	5·4
Hb (g/l)	12·4	2·3	12·2	2·5	12·5	2·2

DRI, dietary reference intake.
* Value was significantly different from that of the normal-folate group ($P<0·05$).
† Data of continuous variables were compared using one-way ANOVA followed by Duncan's multiple range test. The χ^2 test was used for categorical variables.
‡ Serum folate status of individuals was classified into normal (≥14 nmol/l), marginal deficiency (7–14 nmol/l) or deficiency (<7 nmol/l) according to the clinical criteria[23,24].
§ Regular alcohol intake was defined as one or more drinks per week.
‖ Smoking habit was defined as never smoking, or not smoking in the 6 months before the diagnosis of HCC.
¶ Current dietary intake was assessed by 24 h recall. DRI of elderly healthy individuals for folate is 400 μg/d.
†† Habitual folate intake was the dietary intake in the last year, as assessed by a semi-quantitative frequency questionnaire.

consequence of tumour growth. There may be an increased demand for folate as tumours grow. Alternatively, we found that the low serum folate in HCC patients was associated with a low habitual folate intake, suggesting the possible contribution of chronic insufficient dietary folate intake to their low folate status. Whether variations in eating habits and dietary patterns or germline methylenetetrahydrofolate reductase C677T genotype (a marker of long-term folate status) affect the blood folate status of HCC patients is under our investigation.

One of our major findings is that low blood folate status could be a risk factor for tumour progression. After adjustment for age, sex, lifestyle and dietary factors, we found increased risks for tumour progression in large tumours (OR 7·1; 95 % CI 2·27, 21·9), tumour multiplicity (OR 3·2; 95 % CI 1·07, 3·51) and metastasis (OR 4·5; 95 % CI 1·11, 18·4) in HCC patients with deficient blood folate levels. The finding is consistent with results from the folate/methyl-deficient rat model of hepatocarcinogenesis showing that a methyl-deficient diet promoted tumour progression in rat liver[18–20]. Chronic dietary folate/methyl deficiency is known to result in genomic DNA strand breakage[14], uracil misincorporation, hypomethylation within the *p53* tumour suppressor gene[14–16] and altered expression of genes involved in cell-cycle regulation[17], all of which contribute to *in vivo* carcinogenesis[18] and possibly to multiple tumour development. Genome-wide hypomethylation and decreased expression of tumour suppressor genes (*p53* and *p16^{INK4A}*) were observed in early pre-neoplastic liver tumours in rats fed a folate/methyl-deficient diet[28,29] and in patients with HCC[30,31], and may deregulate apoptotic and proliferation processes[32] to favour the development of large tumours. Our data together with results of folate-deficient animal studies support the hypothesis that HCC progression may be associated with low blood folate status. However, the possibility of reverse causality cannot be excluded in the present study given the fact that serum folate decreased as HCC stage progressed, independent of dietary folate intake (Table 4). Whether the mechanistic relationships exist between deficient folate levels, large tumours and tumour multiplicity in human HCC, as proposed in rodent models of hepatocarcinogenesis, will depend on future research results.

It is notable that adjustment for liver injury (ALT levels), tumour proliferation (α-fetal protein levels) and tumour staging, however, negated the effect of folate on the increased risk of advanced tumour progression. The data suggest that clinical lesions present in HCC patients are important confounders that modulate the effect of low folate status in advanced HCC progression. Although the mechanisms that promote human HCC malignancy remain unclear, cancer metastasis is thought to involve complex multi-steps including growth, angiogenesis, dissemination, invasion and survival of cancer cells[33,34]. In response to the *in vivo* microenvironment, differential changes of the primary tumours including

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Table 3. Hepatic injuries, clinical complications and tumour progression by serum folate status of patients with hepatocellular carcinoma‡
(Mean values and standard deviations)

	Serum folate levels§					
	Deficiency (<7 nmol/l)		Marginal deficiency (7–14 nmol/l)		Normal (>14 nmol/l)	
Variables	Mean	SD	Mean	SD	Mean	SD
Glutamic-oxaloacetic transaminase (U/l)	162	143	133	140	101	60
Alanine aminotransferase (U/l)	93	119	94	81	100	67
Cirrhosis						
n	9		31		26	
%	64		78		72	
Ascites						
n	7		15		8	
%	50		38		22	
Portal vein thrombosis						
n	5		8		8	
%	36†		20		22	
α-Fetal protein (μg/l)	1137*	3838	125	420	8	28
Tumour size (cm)	10·0*	14·3	5·8	3·4	3·9	2·7
Tumour number (*n*)	3·0*	0·6	2·4	0·8	2·2	1·0
Metastasis						
n	9		7		4	
%	64*		18		11	

* Value was significantly different from that of the normal-folate group ($P<0·05$).
† Value was significantly different from that of the marginal folate-deficient group ($P<0·05$).
‡ Data of continuous variables were compared using one-way ANOVA followed by Duncan's multiple range test. The χ^2 test was used for categorical variables.
§ Serum folate status of individuals was classified into normal (≥14 nmol/l), marginal deficiency (7–14 nmol/l) or deficiency (<7 nmol/l).

a mixture of tumour cells, stromal cells and endothelial cells may play a role in tumour progression to metastasis[35]. It has been proposed that the degree of viral hepatitis-mediated liver damage may affect intrahepatic metastasis[36]. Thus, changes of the *in vivo* clinical microenvironment in HCC appear to work together and override the effects of folate deficiency on advanced HCC progression. Future studies with larger sample sizes are warranted to confirm this result and to further elucidate the combined effects of low blood folate level, liver injury and tumour behaviour in the cascade of events driving HCC malignancy.

Our findings should be interpreted in the context of a few limitations. The most important one is the relatively small sample size, which reduces the statistical power for subgroup analysis. Insufficient statistical power in multivariate analysis may provide only pilot results. The second is the possibility of error associated with dietary assessments using a 24 h recall or FFQ. Participants may misreport their daily intake or underestimate portion sizes, particularly when ill. Finally, the inherent limitations associated with retrospective study designs cannot conclude folate insufficiency as a cause of advanced HCC progression.

Table 4. Folate status and clinical factors in relation to hepatocellular carcinoma (HCC) stages†
(Mean values and standard deviations)

	HCC stages‡							
	Stage I (*n* 23)		Stage II (*n* 29)		Stage III (*n* 18)		Stage IV (*n* 20)	
Variables	Mean	SD	Mean	SD	Mean	SD	Mean	SD
BMI (kg/m^2)	24·4	2·2	24·2	2·1	24·6	1·5	22·2*	2·5
Current folate intake (μg/d)§	211	110	177	151	188	199	161	109
Habitual folate intake (μg/d)‖	278	127	328	200	330	295	213	110
Serum folate levels (nmol/l)	24·5	11·5	17·3	5·7	13·4	2·3	10·3*	3·3
Albumin levels (g/l)	37·3	0·6	32·5	0·6	33·8	0·5	35·3	0·8
Hb levels (g/l)	12·3	2·8	12·5	1·9	12·7	2·4	11·7	2·3
Glutamic-oxaloacetic transaminase levels (U/l)	108	154	131	94·6	113	85·6	145	121
Alanine aminotransferase levels (U/l)	83·7	59·5	122·2	93·9	77·4	59·9	90·4	98·8
α-Fetal protein levels (μg/l)¶	1·6	1·0	1·7	0·9	2·4	1·7	3·1*	1·8

* Mean value was significantly different from that of the stage I group ($P<0·05$).
† Statistical difference was determined by one-way ANOVA followed by Duncan's multiple range test.
‡ HCC staging was classified according to recognised criteria of the tumour, regional lymph node, and metastases (TNM) system. Detailed descriptions are given in Materials and methods.
§ Current dietary intake was assessed by 24 h recall.
‖ Habitual folate intake was the dietary intake in the last year, as assessed by a semi-quantitative frequency questionnaire.
¶ Data of α-fetal protein levels were log-transformed.

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Table 5. Risk of hepatocellular carcinoma (HCC) progression by blood folate level
(Odds ratios and 95 % confidence intervals)

			HCC progression†					
	Tumour progression		Model A‡		Model B§		Model C‖	
Serum folate levels	Tumour size ≥ 5 cm	Tumour size < 5 cm	OR	95 % CI	OR	95 % CI	OR	95 % CI
Folate ≥ 14 nmol/l	8	29		1·0		1·0		1·0
< 14 nmol/l	34	19	7·1	2·2, 21·9	5·9	1·8, 19·3	3·1	0·91, 10·7
P				0·0007*		0·003*		0·07
Multiple tumours	≥2	1						
Folate ≥ 14 nmol/l	23	14		1·0		1·0		1·0
< 14 nmol/l	44	9	3·2	1·07, 3·51	3·9	1·1, 13·6	2·6	0·68, 9·79
P				0·004*		0·03*		0·16
Metastasis	Yes	No						
Folate≥ 14 nmol/l	4	33		1·0		1·0		1·0
< 14 nmol/l	16	37	4·5	1·1, 18·4	4·6	0·75, 29·1	1·9	0·24, 14·6
P				0·03*		0·10		0·54

* Statistically significant at $P<0·05$.
† Advanced tumour progression is defined as tumour size ≥ 5 cm, tumour number ≥ 2 and metastasis development.
‡ Model A: adjusted for age, sex, alcohol intake and habitual folate intake.
§ Model B: adjusted for all parameters in model A with the addition of albumin, alanine aminotransferase levels and portal vein thrombosis.
‖ Model C: adjusted for all parameters in model B with the addition of α-fetal protein levels and tumour stages.

Despite these limitations, our data provide several implications for HCC prognosis. For clinical treatment of advanced HCC, hepatic resection followed by intrahepatic arterial chemotherapy using the antifolate drug 5-fluorouracil may place more metabolic pressure on the unidentified folate deficiency of HCC patients[37,38]. Persistent folate deficiency during the clinical course of HCC could aggravate nutrition problems if left untreated. Further longitudinally designed and interventional studies with a large sample size may help determine the optimal strategies to improve low folate status of HCC patients and to explore the possible interactions with combined clinical conditions for better prognosis.

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