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Obesity and Microvascular Invasion in Hepatocellular Carcinoma

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Abstract

Background—We hypothesized that hepatocellular carcinoma (HCC) patients with higher Body Mass Index (BMI) might have more microvascular invasion (MVI) in their tumors.

Methods—Records from 138 consecutive patients who underwent surgery at Columbia University Medical Center from January 1, 2002 to January 9, 2008 were evaluated.

Results—40 patients (29%) had MVI, including 14% with BMI <25, 31% with BMI = 25–30, and 40% with BMI >30 ($p = .05$). However, only maximum alpha-fetoprotein was significantly associated with overall mortality in a Cox model.

Conclusions—MVI was associated with obesity. A better understanding of the mechanism of this association may lead to interventions for the treatment and prevention of HCC.

Keywords

Hepatoma and hepatoblastoma; Prognostic studies; Liver and biliary system cancer; Growth factors and receptor; Angiogenesis

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most rapidly increasing causes of cancer deaths in the United States (1). Although many risk factors for HCC are clearly defined, most series show that 5–30% of HCC patients lack a readily identifiable risk factor for their

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DECLARATION OF INTEREST

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cancer (2). The majority of these “cryptogenic” HCC cases in the United States are attributed to nonalcoholic fatty liver disease (NAFLD) (3), a hepatic manifestation of the metabolic syndrome.

Metabolic syndrome is a constellation of medical problems, including obesity, dyslipidemia, diabetes, and insulin resistance (4, 5). Almost one-quarter of the US population meet criteria for the metabolic syndrome (6), and US obesity rates (BMI > 30 kg/m²) also exceed 25% in most regions of the country. Up to three-quarters of obese adults will develop fatty liver disease. Obesity is one of the main causes of NAFLD in the United States, and it accounts for the majority of abnormal aminotransferase measurements seen in this country (7). While the epidemic of cirrhosis from hepatitis C is estimated to peak in 2010, the obesity/metabolic syndrome epidemic shows no signs of abating (5, 8).

NAFLD includes a spectrum of disorders, from fatty liver disease to progressive inflammation and cirrhosis. Ultrasound and MRI studies from the United States and other Western countries suggest that 20–30% of the population have evidence of NAFLD (9–11). About 10% of patients with NAFLD progress to non-alcoholic steatohepatitis (NASH), and up to a quarter of those with NASH progress to cirrhosis (12). Retrospective data suggest that after development of cirrhosis, up to a quarter of those with NASH develop HCC (13). These figures lead to theoretical HCC incidences of 0.6 per 100,000 to 210 per 100,000 persons. The obesity/metabolic syndrome epidemic is relatively recent, and it is likely that several decades are required before NASH develops into cirrhosis. Thus, the NASH–HCC “epidemic” may not have fully established itself yet.

Although little is known about the relationship between metabolic syndrome and HCC, several lines of evidence link the two conditions. In patients with HCV-related cirrhosis, those with concomitant steatosis have higher incidence rates of HCC than those without fatty changes, and increasing grades of pathological steatosis are associated with increased odds of developing HCC, again suggesting a possible connection between adiposity and cancer (14). Finally, HCC may also develop in the context of obesity without cirrhosis, suggesting a more direct pathway between metabolic syndrome and cancer (15).

Clinically, features of the metabolic syndrome have been associated with worsened HCC outcomes. An article by Calle and colleagues (16) showed that high BMI was associated with significantly increased cancer death rates, particularly from HCC. In a small, hypothesis-generating study of men with NAFLD and HCC, tumors had evidence of hypervascularity on imaging (17).

We hypothesized that one possible reason for the relationship between obesity and worsened outcome in HCC could be increased angiogenesis, as manifested by increased microvascular invasion (MVI) seen in tumor samples (18). One possible mediator for this relationship is leptin. Leptin is increased in patients with obesity (19), and is also related to angiogenesis and HCC development *in vitro* and *in vivo*. For instance, leptin was shown to induce neovascularization in corneas from normal rats but not in corneas from Zucker rats, which lack functional leptin receptors (20). Leptin also led to upregulation of angiogenic cytokines including vascular endothelial growth factor (VEGF) in hepatic stellate cells (21). In another study, Zucker rats did not develop the same degree of neovascularization and VEGF expression as their control littermates, suggesting that leptin exerted a proangiogenic effect in the non-Zucker animals when all were fed a choline-deficient diet. This neovascularization mediated the progression of NASH to HCC in the Zucker animals (22).

We hypothesized that HCC patients with a higher BMI might have more MVI in their tumors. Vascular invasion by tumor cells is a known poor prognostic feature in patients with HCC, as well as with other malignancies (23–26). MVI has been shown to be a poor

prognostic feature in HCC (27–30). For instance, one report evaluated 10-year survival in patients transplanted for HCC in Germany. As patients with macrovascular invasion were not eligible for liver transplant, MVI could be evaluated independently. On multivariable analysis, MVI was independently predictive of overall survival; mortality among patients with MVI was twice as high as among other patients ($p = .04$) (27). In another study of 75 patients who received liver transplants, MVI was the strongest pathological predictor of recurrent HCC (OR = 14, $p < .001$) (30).

METHODS

We retrospectively identified all patients who underwent surgery or resection for HCC in Columbia University Medical Center between January 1, 2002 and September 1, 2008. All patients aged more than or equal to 18 years at the time of surgery, with a pathologic diagnosis of hepatocellular carcinoma and available BMI (BMI is defined as weight in kilograms divided by height in meters squared) were included in the sample. The protocol was approved by the Institutional Review Board of Columbia University Medical Center. In our dataset, out of a total of 205 patients who underwent surgery, 66 were excluded for missing BMI information, and 24 for missing pathology.

Data collection

From paper charts and Columbia's electronic data warehouse, we abstracted age at diagnosis, sex, race/ethnicity (self-reported), maximum alpha-fetoprotein (AFP), risk factors for liver disease, and Child–Pugh score prior to surgery. Height and weight were obtained through either the clinical chart or a United Network for Organ Sharing (UNOS) query. Where more than one set of height and weight measurements was available, we used the measurements dated closest to and preceding surgery. Patients without BMI data were excluded. We categorized BMI as < 25 (normal or underweight), 25–30 (overweight), or > 30 (obese). Child–Pugh score was computed according to the formula incorporating measures of serum total, bilirubin, albumin, international normalized ratio (INR), ascites, and encephalopathy grade taken closest to and preceding the date of surgery (31).

Pathologic examination

All liver specimens were analyzed for MVI on H&E-stained and paraffin-embedded sections used for routine diagnostic purposes, by an attending gastrointestinal pathologist at the Columbia University Medical Center. The MVI was defined as identification of hepatocellular carcinoma tumor cells within endothelial-lined spaces on standard hematoxylin and eosin-stained slides. None of the pathologists was aware of the study hypotheses. All slides with reported MVI were re-reviewed by two pathologists (HR and KD). Cases initially reported as indeterminate for MVI were reviewed and reclassified as positive or negative for MVI. We evaluated steatohepatitis using Kleiner et al.'s criteria (32). When there was more than one tumor present in a specimen, a representative slide was chosen at random. For those patients who had more than one surgery, we chose the most recent specimen available.

Statistical analysis

Chi-square or Fisher exact tests were used to evaluate the statistical significance of differences between patients with respect to the above variables. We then developed a logistic regression model to analyze the association of MVI status with BMI. The multivariable model was built by incorporating features that had been suggested in our univariate analyses to be associated with MVI. Our data suggested that BMI and AFP were significantly different between the two groups; we included tumor size as another variable shown in the literature to be associated with vascular invasion. We then used Kaplan–Meier

survival curves and Cox proportional hazard models to determine risk factors for increased mortality. Survival was determined from the date of diagnosis to the date of death, and 99 people were censored because of lack of death information. All statistical analyses were performed with SAS software (Cary, NC).

RESULTS

The patients' median age was 57 years, and 79% were males. Overall, 68 (50%) were overweight, and 25% were obese. Of 138 patients with HCC, 40 (29%) had MVI, including 14% with BMI < 25, 31% with BMI = 25–30, and 40% with BMI > 30 ($p = 0.05$) (Table 1). AFP and BMI were initially considered as continuous variables. AFP was then dichotomized to above and below the median, and BMI was divided into three groups based on the standard classifications of normal weight, overweight, and obese. The distribution of AFP was as follows: maximum value of 1,459,810 (ng/ml), median 28.1 (ng/ml), and minimum 1.5 (ng/ml). For BMI, the maximum was 46.9 (kg/m²), median 28.1 (kg/m²), and minimum 19.0 (kg/m²). Using a 2×3 contingency table, the odds ratios for the association of BMI with MVI were 2.7 for overweight compared to normal weight patients (95% Confidence Interval (CI) = 0.91–7.87, $p = .07$), and 4.0 for obese compared to normal weight patients (95% CI = 1.25–12.8, $p = .02$). Thus, the higher the BMI, the higher the proportion of patients with MVI. The overall p -value (Chi-squared) was .05, and a Cochran–Armitage test for trend was also significant ($p = .02$).

In a multivariable logistic regression model that included BMI group, tumor size, and median AFP, only AFP more than the median was an independent significant predictor of MVI (Table 2). We used the Hosmer and Lemeshow goodness of fit test to evaluate this model and obtained p -value = .07 (we want p -value to be greater than .05 to not reject the null hypothesis if the model fits the data well). Only six patients had definite evidence of steatohepatitis on pathology using Kleiner et al.'s criteria (32). Twenty patients had some combination of at least moderate steatosis and/or steatohepatitis.

An unadjusted Kaplan–Meier analysis showed that HCC patients with MVI had somewhat poorer overall survival than HCC patients without MVI, but the difference was not statistically significant ($p = .08$, logrank) (Figure 1). Three-year survival for those with MVI was 64%, and 79% for those without MVI ($p = .08$, logrank).

In a Cox proportional hazards model that included risk factors for liver disease, patients with AFP more than or equal to median had a higher all-cause mortality than patients with lower AFP levels (HR = 3.5, $p = .006$, 95% CI = 1.4–8.7). Neither MVI nor BMI was independently associated with all-cause mortality (Table 3). BMI was also not significantly associated with AFP in a univariate Chi-squared analysis ($p = .64$). Interestingly, when we analyzed just the lower AFP group separately for the relationship between MVI and survival, we found a statistically significant hazard ratio of 2.2 for those with MVI ($p = .01$, 95% CI = 1.18–4.02). Patients were censored if they were lost to follow up and we did not find their date of death in the social security death index. In this case the date of last follow-up from the chart was used for the Kaplan–Meier curves.

We excluded 52 patients because they did not have available BMI data. We evaluated these patients for some clinical characteristics to see how they differed from the included patients. We found that the median AFP was significantly higher in patients who were excluded due to missing BMI data compared with the included patients, with a p -value of .02 (Pearson's chi-squared). Median age, presence or absence of MVI, and Child's class were not significantly different between the two groups.

DISCUSSION

For many common cancers, obesity has been associated with both increased risk and poor outcome. HCC is no exception. We show that in patients with HCC, increasing BMI was associated with increasing prevalence of MVI within tumors. While 3-year survival was lower in the patients with MVI, overall survival was not significantly different when controlling for other factors also associated with both MVI and outcome, such as AFP.

We hypothesized that tumors of obese patients might have more MVI in their tumors than those of leaner patients. Adipose tissue induces expression of VEGF and adipokines in both human and animal models (33). Adipokines are cytokines secreted by adipose tissue. Some of these, such as leptin, have been shown to be angiogenic (20). For instance, in patients with renal cell carcinoma, high serum leptin levels were significantly associated with venous invasion in pathology samples and with aggressive clinical features (34).

In addition, vascular invasion in HCC is associated with other markers of angiogenesis, such as VEGF expression (35, 36). Vascular invasion in other cancer types, such as gastric cancer, has been linked to both microvessel density and VEGF staining (37, 38). These data support the plausibility of the hypothesis that HCC, in the setting of increased BMI, may be associated with increased angiogenesis. We are beginning studies to evaluate our specimens for expression of angiogenic markers using immunohistochemistry, and to prospectively measure adipokines, such as leptin, in our HCC patients to try to validate this hypothesis.

In our Cox model, the difference in all-cause mortality between patients with and without MVI was not statistically significant, perhaps because of the small sample size or because AFP, a tumor marker correlated with tumor stage and prognosis, is a more important prognostic feature and “subsumes” MVI. Interestingly, in a subset of patients with lower AFP, those with MVI did have a significant 2-fold increase in mortality. BMI was not independently associated with increased mortality, perhaps because of the large proportion of our patients who were overweight or obese, and because of the collinearity of BMI with MVI. We did not have access to performance status data for most patients, but patients with higher BMI may have had better performance status than those with other risk factors, or may have differed from those with normal or low BMI in some other way associated with better outcome. Despite the relatively high rate of censoring in our study, simulation studies suggest that the influence of censoring of this degree on the bias of the estimate is minimal (39).

Nearly 25% of the US population currently meet criteria for the metabolic syndrome (6), and in most regions of the country more than 25% are obese. Up to three-quarters of obese adults will develop fatty liver disease, and HCC from obesity may also continue to increase for many decades (8). If we can better understand the role of metabolic syndrome in cancer outcomes, we may be able to develop better treatment options for patients with this condition. For instance, if further studies show that the tumors of patients with a higher BMI have more vascular invasion than the tumors of leaner patients, we might analyze trial data to see if obese patients respond better than leaner patients to anti-angiogenic agents.

An important limitation of our study is its relatively small sample size, which prevented us from including a number of variables in our logistic regression models that may be associated with obesity, MVI, and mortality. Another limitation is that we relied on chart data collected retrospectively. A related limitation is that we were unable to obtain data on other variables, including performance status and ascites.

We also excluded 52 patients who did not have available BMI data. When we evaluated these patients, we found that the median AFP was significantly higher in the excluded

patients than the included patients ($p = .02$). Since AFP was already the strongest predictor of MVI and mortality, we didn't expect that these excluded patients would significantly affect our overall results. It is possible that if all the excluded patients had very low (or high) BMIs, the relationship with MVI might have changed.

Another limitation to our data was that our pathology slides were not all centrally reviewed, although we did review a subset of cases to ensure consistency. When there was more than one tumor present in a specimen, we were not able to tell which specimen we were selecting from a particular case, and sampling error could have led to inconsistent results.

In summary, we found a significant relationship between increasing BMI and the presence of MVI in pathology specimens in a cohort of 138 patients who underwent surgery for HCC. However, neither BMI nor MVI was a significant independent predictor of all-cause mortality in our patients. Because of the obesity epidemic in this country, it is crucial to begin to study the pathologic and biologic correlates of obesity and other features of metabolic syndrome in relation to cancer outcomes. These studies may have implications for more effective prevention and treatment of HCC.

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Figure 1.
Kaplan-Meier Survival Curve in Patients With and Without MVI.

Table 1
Subject and Tumor Characteristics in Patients With and Without Microvascular Invasion (MVI)

Characteristic	MVI (+)		MVI (–)		Total		p-value	Missing
	(n)	(%)	(n)	(%)	(n)	(%)		
Age	40	29	98	71	138	100		
57	16	23	53	77	69	50	.13	0
<57	24	35	45	65	69	50		
Gender							.27	0
Male	34	31	75	69	109	79		
Female	6	21	23	79	29	21		
Race/ethnicity							.3	2
White	25	32	54	68	79	58		
Black	2	11	16	89	18	13		
Hispanic	9	36	16	64	25	18		
Asian	4	27	11	73	15	11		
Child's class							.8	2
1	17	26	49	74	66	48		
2	13	31	29	69	42	31		
3	9	31	20	69	29	21		
Tumor median							.67	
3 cm	15	25	45	75	60	55		30
<3 cm	14	29	35	71	49	45		
AFP median							.01	8
28	25	38	40	62	65	50		
<28	12	18	53	82	65	50		
HCV							.53	2
(+)	25	27	66	73	91	66		
(–)	15	33	31	67	46	33		
HBV							.42	2
(+)	8	36	14	64	22	16		
(–)	32	28	83	72	115	84		

Characteristic	MVI (+)		MVI (-)		Total		p-value	Missing
	(n)	(%)	(n)	(%)	(n)	(%)		
ETOH							.23	2
(+)	13	37	22	63	35	26		
(-)	27	26	75	74	102	74		
Other causes							.32	2
(+)	0	0	5	100	5	4		2
(-)	40	30	92	70	132	96		2
HTN							.56	3
(+)	14	26	40	74	54	40		
(-)	25	30	57	70	82	60		
Hyperlipidemia							.47	1
(+)	4	40	6	60	10	7		
(-)	36	28	92	72	128	93		
Diabetes							.25	1
(+)	15	36	27	64	42	30		
(-)	25	26	71	74	96	70		
BMI							.05	1
<25	5	14	30	86	35	25		
25-30	21	31	47	69	68	50		
>30	14	40	21	60	35	25		
Steatosis							.08	1
(+)	9	45	11	55	20	15		
(-)	31	26	87	74	118	85		

Table 2

Logistic Regression of Predictors of Microvascular Invasion (MVI)

Variable	OR	95% CI		p
Tumor size	0.43	0.16	1.24	.12
AFP	3.33	1.15	9.62	.03
BMI 25–30	1.91	0.5	7.39	.35
BMI >30	1.67	0.37	7.52	.5

All variables adjusted for each other.

Table 3

Cox Proportional Hazards Model of Predictors of All-Cause Mortality

Variable	OR	95% CI
Age group		
57	1	Referent
<57	0.6	0.2–1.6
Child–Pugh score		
1	1	Referent
2	0.3	0.1–1.2
3	1.7	0.6–4.5
AFP		
<28	1	Referent
28	3.5	1.4–8.6
Hepatitis C		
No	1	Referent
Yes	0.8	0.2–2.4
Hepatitis B		
No	1	Referent
Yes	0.5	0.1–1.8
Ethanol use		
No	1	Referent
Yes	1.2	0.5–3.1
MVI		
No	1	Referent
Yes	1	0.4–2.7
BMI		
<25	1	Referent
25–30	0.6	0.2–1.7
>30	0.7	0.2–2.1

All variables are adjusted for each other.