World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v16.i24.3025

World J Gastroenterol 2010 June 28; 16(24): 3025-3032
ISSN 1007-9327 (print)

BRIEF ARTICLE

Glycated hemoglobin and antidiabetic strategies as risk factors for hepatocellular carcinoma

Valter Donadon, Massimiliano Balbi, Francesca Valent, Angelo Avogaro

Valter Donadon, Massimiliano Balbi, Department of Medicine, Internal Medicine 3rd, Pordenone Hospital, Via Montereale 24, Pordenone 33170, Italy
Francesca Valent, Institute of Hygiene and Epidemiology, Udine University Hospital, Udine 33100, Italy
Angelo Avogaro, Department of Clinical and Experimental Medicine, Padova University, Padova 35100, Italy
Author contributions: Donadon V designed the research; Balbi M and Donadon V performed the research; Valent F analyzed the data; Donadon V, Balbi M and Avogaro A wrote the paper.
Correspondence to: Dr. Valter Donadon, Department of Medicine, Internal Medicine 3rd, Pordenone Hospital, Via Montereale 24, Pordenone 33170, Italy. valter.donadon@gmail.com
Telephone: +39-434-399330 Fax: +39-434-399559
Received: March 12, 2010 Revised: April 17, 2010
Accepted: April 24, 2010
Published online: June 28, 2010

Abstract

AIM: To evaluate the relationship between glycemic control [assessed by glycated hemoglobin (HbA1c)], antidiabetic therapies and the risk of hepatocellular carcinoma (HCC).

METHODS: We recruited 465 patients with HCC, 618 cases with liver cirrhosis and 490 controls with no liver disease. Among subjects with type 2 diabetes mellitus (DM2), the associations between the antidiabetic strategies and HbA1c level with HCC were determined through 2 series of multivariate logistic regression models using cirrhotic patients and controls as comparison groups.

RESULTS: DM2 prevalence was 31.2% in patients with HCC, 23.2% in cirrhotic patients and 12.6% in controls ($P < 0.0001$). In 86% of study subjects, DM2 had been diagnosed for more than 1 year before the HCC diagnosis. HCC patients with DM2 had a 1.5-2.5-fold increased risk of liver cancer. The HbA1c mean levels were significantly higher in DM2 patients with HCC than in cirrhotic and control DM2 patients. Antidiabetic treatment with metformin was more common among cirrhotic and control DM2 subjects than among cases with HCC. In both series of multivariate analyses, treatment with metformin significantly reduced the risk of HCC by more than 80% compared with sulphonylureas and insulin therapy. No significant differences were seen between sulphonylureas and insulin treatment. Elevated HbA1c levels were positively related to the risk for HCC in diabetic patients, with a 26%-50% increase in risk for each 1% increase in HbA1c values.

CONCLUSION: In patients with preexisting DM2, the risk of HCC is positively associated with poor chronic glycemic control and significantly decreased by metformin therapy.

Key words: Hepatocellular carcinoma; Type 2 diabetes mellitus; Glycemic control; Metformin therapy; HbA1c level

Peer reviewers: Yasuji Arase, MD, Department of Gastroenterology, Toranomon Hospital, 2-2-2 Toranomonminato-ku, Tokyo 105-8470, Japan; Ned Snyder, MD, FACP, AGAF, Professor of Medicine, Chief of Clinical Gastroenterology and Hepatology, Department of Internal Medicine, The University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-0764, United States

Donadon V, Balbi M, Valent F, Avogaro A. Glycated hemoglobin and antidiabetic strategies as risk factors for hepatocellular carcinoma. *World J Gastroenterol* 2010; 16(24): 3025-3032 Available from: URL: http://www.wjgnet.com/1007-9327/full/v16/i24/3025.htm DOI: http://dx.doi.org/10.3748/wjg.v16.i24.3025

INTRODUCTION

The incidence and mortality rates of hepatocellular carci-

noma (HCC) have significantly increased in recent years, particularly in Western countries[1,2]. The main known risk factors for HCC are hepatitis C and B virus (HCV, HBV) infections and chronic alcohol abuse, but at least 25% of HCC cases do not have any recognized etiology. Diabetes mellitus has been proposed to be associated with HCC development[3], and several investigations in recent years clearly indicated that type 2 diabetes mellitus (DM2) is a risk factor for HCC[1,4-9]. During the past 2 decades, the prevalence of diabetes mellitus, and in particular of DM2, has dramatically increased in many countries, including Italy[10]. Sedentary lifestyles, excessive food consumption and obesity appear to be the main causes of the current diabetes mellitus epidemic[11].

It is unclear how DM2 influences hepatocarcinogenesis. Insulin resistance (IR) is a basic feature of DM2, a disorder characterized by hyperglycemia associated with IR and consequent hyperinsulinemia. Several reports have suggested that the mechanism underlying the effects of DM2 and antidiabetic therapies on hepatic carcinogenesis could be related to IR and hyperinsulinemia[12-16]. In DM2 patients, insulin plasma levels are chronically increased by therapies based on both exogenous insulin or insulin secretagogues, such as sulphonylureas. An excess of insulin plasma levels is the proposed mechanism underlying the well established association of DM2[16-18] and obesity[19] (the so called diabesity) with several types of solid tumors[20,21]. In a previous study, we found that DM2 in our population was an independent risk factor for HCC, and that it precedes HCC development[12]. In addition, in male chronic liver disease (CLD) patients with DM2, the risk of HCC is increased by insulin or sulphonylurea treatment. We also reported that the association between IR and CLD began in the early stages of liver fibrosis, and that DM2 significantly increased IR in HCC patients. Therefore, IR together with the consequent hyperinsulinemia, seems to play a major role in the link between DM2 and hepatocarcinoma[13].

Recent reports have shown that liver carcinogenesis is increased not only in HCV infected patients with previously diagnosed and/or treated DM2[22,23], but also in HCV infected subjects with no pre-existing history of DM2 and in the early stages of glucose intolerance, as diagnosed by a 75 g oral glucose tolerance test[24]. In addition, , a positive association between high dietary glycemic load and risk of HCC in patients with chronic HBV and HCV infection has been reported[25]; the pathogenesis and outcome of cryptogenic HCC seems to be more closely associated with the risk factors for metabolic syndrome than with HBV and HCV[26].

Despite extensive literature linking glucose intolerance with hepatocarcinogenesis, it remains unclear whether blood glucose control may influence the malignant potential that DM2 exerts on the liver. Only one prospective study reported that hyperglycemia was associated with increased total cancer risk in women and men, independently of obesity[27]. As far as we know, the effect of glycemic control on liver carcinogenesis and the role of treatment strategies to achieve the metabolic control in DM2 patients with CLD have not been investigated yet.

Therefore, the aims of our study were to explore the association between HCC and DM2 in CLD subjects, and to assess the relationships between glycemic control, as determined by glycated hemoglobin (HbA1c) measurement, antidiabetic strategies and the risk of HCC in DM2 patients with CLD.

MATERIALS AND METHODS

Ethics

This work has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. All of the studies were performed retrospectively by collecting and analyzing data from the patient records. This study had been approved by the Institutional Review Board of our hospital.

Design and case selection

For this retrospective, hospital based case-control study, subjects were recruited from patients attending the 3rd Internal Medicine of Pordenone General Hospital (Pordenone, Italy). The 3rd Internal Medicine is a tertiary referral center for liver diseases and diabetes mellitus. The case group consisted of patients with incident HCC admitted to the hospital. We then recruited two comparison groups: the first consisted of patients admitted for liver cirrhosis (LC); the second consisted of control individuals admitted for a wide spectrum of acute conditions other than diabetes mellitus and liver disease as primary diagnoses.

HCC group

The HCC group consisted of 465 (364 male, 101 female) consecutive patients with newly diagnosed HCC attending the 3rd Internal Medicine from January 1994 to June 2006. Of these, 398 (85.6%) were diagnosed by cytological or histological examination of hepatic focal lesions; the remaining 67 (14.4%) were diagnosed according to the following acknowledged criteria[28]: ultrasound examination (also by using micro-bubbles of sulfur hexafluoride as contrast dye in the last 3 years), α-fetoprotein (AFP) > 400 ng/mL, computerized tomography liver scan and/or magnetic resonance imaging. Based on the type of presentation, the HCC patients were subdivided into two groups: (1) 305 subjects from our surveillance program of HCC in cirrhotic patients (follow-up group: FU); and (2) 160 subjects presenting with symptomatic and advanced neoplastic disease of the liver (clinically overt: CO). The FU group included patients with a small single hepatic tumor who received the diagnosis within the HCC surveillance program in cirrhotic patients based on ultrasound examinations and AFP determinations every 3-6 mo; the CO group included cases with advanced, large size and symptomatic HCC at diagnosis. Clinical data, biochemical parameters and the antidiabetic treatment of patients were considered at the time of HCC diagnosis during the first admission to our Hospital.

In order to gain complete and accurate information on the time interval between DM2 onset and the diagnosis of HCC and to determine the levels of HbA1c testing before the HCC diagnosis, we reviewed, starting from October 1985, all medical documentation stored at the Diabetes Clinic.

Liver cirrhosis group

We enrolled 618 patients (450 male, 168 female) with LC, by selecting from 3560 cirrhotic patients treated at our Department subjects frequency-matched with the HCC cases, according to age (± 5 years), gender, body mass index (BMI), transaminases, serologic markers of HBV and HCV infections, alcohol consumption, time of hospital admission. The diagnosis of cirrhosis was performed either by hepatic biopsy or by ultrasound examination in the fasting state, showing the presence of splenomegaly, hypertrophy of the left or caudal lobes and surface irregularity or by transient elastography using the Fibroscan (Echosens, Paris) with a liver stiffness > 12.5 kPa[29]. LC patients were admitted to our Hospital for diagnosis, staging or therapy of LC. Clinical data, biochemical parameters and antidiabetic treatment were considered at the time of the first admission to our Hospital. According to Child's classification of cirrhosis, patients were classified as follows: class A: 55.5%; B: 24.3% and C: 20.2%. In cirrhotic patients, the presence of HCC was ruled out through ultrasound examinations, computed tomography or magnetic resonance imaging of the upper abdomen and AFP determination.

Control group

We enrolled 490 control subjects (385 male, 105 female) as follows: from 28740 inpatients of our hospital, we generated a pool of 7610 subjects with available tests as possible controls. Then we selected from them a frequency-matched subject for each HCC or LC patient, according to age (± 5 years), gender, BMI, time of hospital admission. Clinical data, biochemical parameters and therapeutic schedule of control subjects were considered at the time of the first admission to our Hospital. Subjects admitted for malignancies, alcohol-related disease, liver disease and DM2 as primary diagnoses were excluded from the study. The primary diagnoses of admission were: chronic heart failure (34.9%), hypertension (21.4%), chronic obstructive broncho-pneumopathies or pneumonia (16.5%), atrial fibrillation (7.8%), deep venous thrombosis (6.5%), fever of unknown origin (5.3%), benign tumors (4.1%), gastritis (3.5%). These subjects were assumed to represent our Region's general population as regards the prevalence of HCV, HBV infection, alcohol consumption and DM2. The prevalence of these parameters[30-33] in the free living population of the Pordenone area with an age range of 60-75 years and a similar age sub-sample of our control group is reported in Table 1.

Table 1 Mean prevalence of HCV infection, HBV infection, alcohol abuse and DM2 in the free living population of the Pordenone area and controls in this study

	General population (%)		Control group of the study (%)
	Global	60-75 yr age interval	
HCV positive	3.2	5.0	5.3
HBV positive	1.2	1.7	1.2
Alcohol abuse	4.5	5.0	4.7
DM2	4.8	12.4	12.6

HCV: Hepatitis C virus; HBV: Hepatitis B virus; DM2: Type 2 diabetes mellitus.

Methods

DM2 was diagnosed using the American Diabetes Association criteria[34]. Biochemical parameters were determined at the Pordenone Hospital central laboratory, using standardized and validated methods. Venous blood samples were taken in the morning after 12 h overnight fasting. Blood samples were available for 460 HCC cases and for all LC patients and controls. HbA1c was measured by high performance liquid chromatography (A1cHA-8160 Menarini, Italy)[35] for all diabetic subjects in our study. The nondiabetic range for our method of HbA1c testing was 4.0%-6.0% using a DCCT (Diabetes Control Complications Trial)-based assay[36]. For each subject we used the mean of 2 samples, taken on 2 consecutive days at the time of enrollment.

To assess the chronic diabetes controls in HCC patients with pre-existing DM2, we evaluated the individual HbA1c values tracked in the time before liver cancer diagnosis. Based on the records of the Diabetes Clinic, we determined the mean HbA1c levels of at least 3 tests/patient carried out approximately every 2 years before HCC clinical occurrence.

HBV surface antigen (HBsAg), anti-HBV surface antigen (anti-HBs), anti-HBV core antigen (anti-HBc), and hepatitis B e antigen (HBeAg) were determined using commercial assays (Abbott Diagnostic Division, Wiesbaden, Germany). Sera were also screened for antibodies against HCV (anti-HCV) using a third-generation microparticle enzyme immunoassay (AxSYM HCV version 3.0, Abbott Diagnostic Division). Positive samples were tested for anti-HCV using a third-generation line immunoassay (Immunogenetics, Gent, Belgium) and for serum HCV-RNA using the Roche Amplicor version 2.0 (Roche Molecular System, Pleasenton, CA, USA).

Information on consumption of alcoholic drinks was collected through a structured questionnaire which was returned by 455 HCC patients and by all LC and control subjects. Average alcohol content was estimated as 5% for beer, 12% for wine and 40% for spirits[37]. Alcohol abuse was defined as a daily consumption of over 30 g for males and over 20 g for females.

Statistical analysis

All the data were collected in a computerized database. Normality of the distributions of all continuous variables was tested by the Shapiro-Wilk test. The Student *t*-test and ANOVA were used to assess the statistical significance of

Table 2 Characteristics of patients in the HCC, LC and control groups *n* (%)

	HCC *n* = 465	LC *n* = 618	Controls *n* = 490	*P*
Sex, male	364 (78.3)	450 (72.8)	385 (78.6)	0.1706
Alcohol abuse	233 (51.2)	312 (50.6)	23 (4.7)	< 0.0001
DM2	145 (31.2)	144 (23.2)	62 (12.6)	< 0.0001
ALT ⩾ 53 IU/L	113 (24.3)	150 (24.3)	N/A	0.9911
HBV positive	39 (8.4)	39 (6.3)	N/A	0.1908
HCV positive	268 (57.6)	285 (46.1)	N/A	0.0002

P-value of χ^2 test. HCC: Hepatocellular carcinoma; LC: Liver cirrhosis; N/A: Not available.

Table 3 Association of DM2 with HCC: results of multivariate analyses using controls and LC subjects as comparison groups

	OR	95% CI	*P*
Control group			
Age ⩾ 65 yr	0.873	0.606-1.259	0.4675
BMI ⩾ 25 kg/m^2	1.048	0.769-1.426	0.7681
Alcohol abuse	22.069	13.749-35.425	< 0.0001
DM2	2.507	1.703-3.692	< 0.0001
LC group			
Age ⩾ 65 yr	2.457	1.855-3.252	< 0.0001
BMI ⩾ 25 kg/m^2	1.560	1.197-2.032	0.0010
HBV positive	2.372	1.363-4.127	0.0022
HCV positive	2.783	1.896-4.084	< 0.0001
Alcohol abuse	1.937	1.318-2.845	0.0008
ALT ⩾ 53 IU/L	0.919	0.672-1.256	0.5951
DM2	1.456	1.072-1.979	0.0162

BMI: Body mass index; OR: Odds ratio; 95% CI: 95% confidence interval; ALT: Alanine transaminase.

differences among groups for continuous variables with normal distribution; the Wilcoxon rank sum and Kruskal-Wallis tests were used for continuous variables with non-normal distribution. The χ^2 test was used to evaluate the significance of differences among groups for categorical variables. Multivariate logistic regression was used to assess the association between HCC and DM2, antidiabetic therapy, and glycemic control after adjusting for potentially confounding factors. Separate models were built considering LC patients and controls as the comparison groups. When assessing the association of DM2 with the risk of HCC using LC patients as the control group, we considered sex, age (⩾ 65 years *vs* < 65 years), BMI (⩾ 25 kg/m^2 *vs* < 25 kg/m^2), HBV and HCV infection, alcohol abuse, alanine transaminase (ALT) level (> 53 IU/L *vs* ⩽ 53 IU/L); when we used control subjects as the comparison group, potential confounders included only sex, age, BMI, and alcohol abuse. When evaluating which DM2 characteristics were associated with risk of HCC among diabetics, covariates included sex, age, BMI, HBV and HCV infection, alcohol abuse, ALT level, triglycerides, cholesterol, type of antidiabetic therapy (metformin, sulphonylureas, insulin), HbA1c levels (continuous and in categories using the following cut-offs: 6.5%, 7.5%, 8.5%), DM2 duration (continuous and using the following 3 cut-offs: 5, 10, 15 years) when we used LC patients as the comparison group and sex, age, BMI, HBV and HCV infection, alcohol abuse, ALT level, triglycerides, cholesterol, type of antidiabetic therapy, HbA1c levels, and DM2 duration when we used the controls. Among HCC cases, HbA1c levels at the time of cancer diagnosis were compared with an average of at least 3 tests carried out approximately every 2-3 years before the diagnosis of HCC. The Wilcoxon signed rank test and Spearman's correlation coefficient (*r*) were used to compare the paired differences and the kappa statistic to evaluate concordance of categories. Since at enrollment DM2 duration was either unknown or < 12 mo duration in 14% of diabetic subjects and 1-2 years duration in an additional 8%, in the study of the association between DM2 and HCC we performed a sensitivity analysis considering as non-diabetic subjects those whose diagnosis of DM2 occurred in the 12, 24, 60, and 120 mo before enrollment to allow for adequate induction time. For the same reason, we performed a sensitivity analysis excluding from the analyses regarding diabetes therapy and glycemic control, those patients with diagnosis of DM2 in the 12, 24, 60, and 120 mo before enrollment. All *P*-values < 0.05 were considered statistically significant. Analyses were conducted with SAS v9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Demographic characteristics of the three groups studied, and exposures relevant to liver diseases are illustrated in Table 2. The prevalence of DM2 was significantly higher ($P < 0.0001$) in HCC patients (31.2%) than in the LC group (23.2%) and in controls (12.6%). Using control subjects as a comparison group, after adjusting for age, BMI and alcohol abuse, DM2 significantly increased the odds ratio (OR) of HCC by 2.5 times (Table 3). Using LC patients, after adjusting for sex, age, BMI, alcohol abuse, HBV and HCV infection, and ALT level, DM2 significantly increased the OR of HCC by 1.5 times (Table 3). Alcohol abuse was the strongest predictor of HCC using controls subjects as comparison; it also increased the risk of HCC, as did HBV and HCV infections by comparing HCC with LC patients.

Table 4 illustrates the levels of BMI, cholesterol, triglycerides, and HbA1c, in HCC, LC and control subjects with DM2. BMI, cholesterol and triglycerides were not different between the three groups of subjects studied. The HbA1c levels evaluated at the time of enrollment in the study were significantly higher in HCC patients (7.5%, with 8.1% ± 1.8% in FU and 6.3% ± 1.1% in CO subgroups $P < 0.0001$) than in controls (6.8%) and in LC patients (6.6%). Among HCC diabetic cases, current HbA1c levels were on average only 0.2 percentage points lower than the average value of 3 previous values that we assumed to be representative of glycemic control in the past years. Current and average past HbA1c levels were highly correlated ($r = 0.77$, $P < 0.0001$) and agreement between categories (using 6.5%, 7.5%, and 8.5% as the cut-offs) was moderate to good, as expressed by the weighted kap-

Table 4 Biochemical characteristics of diabetic HCC, LC patients and controls, DM2 duration and therapy (mean ± SD)

	HCC (n = 145)	LC (n = 144)	Controls (n = 62)	P
BMI (kg/m^2) (median)	25.6 ± 2.9 (26.0)	25.8 ± 3.8 (25.0)	25.1 ± 2.4 (25.3)	0.3175[a]
Cholesterol (mg/dL) (median)	155.4 ± 47.4 (152)	155.9 ± 37.7 (155)	160.6 ± 45.4 (173.0)	0.6013[a]
Triglycerides (mg/dL) (median)	110.6 ± 60.7 (94.5)	108.0 ± 45.5 (101.5)	111.5 ± 67.9 (95.0)	0.8060[a]
Fasting plasma glucose (mg/dL) (median)	126.7 ± 47.3 (110.5)	109.3 ± 32.9 (100.0)	98.0 ± 20.6 (95.0)	< 0.0001[a]
Current HbA1c (%) (median)	7.5 ± 1.8 (7.3)	6.6 ± 1.5 (6.4)	6.8 ± 1.5 (6.5)	0.0001[a]
Average past HbA1c (%) (range)	7.7 ± 1.5 (4-13.5)	N/A	N/A	
DM2 duration (mo) (median)	141.6 ± 81.0 (146)	135.4 ± 98.3 (117)	124.9 ± 100.5 (119)	0.2496[a]
Therapy, n (%)				< 0.0001[b]
Not reported	0 (0)	12 (8.3)	2 (3.2)	
Metformin	13 (9.0)	39 (27.1)	15 (24.2)	
Sulphonylureas	68 (46.9)	33 (22.9)	32 (51.6)	
Insulin	64 (44.1)	60 (41.7)	13 (21.0)	

[a]P of Kruskal-Wallis test; [b]P of Fisher's exact test. HbA1c: Glycated hemoglobin A1c.

pa 0.5456. Mean HbA1c levels were not different among HCC patients with or without HBV, HCV and alcohol abuse (data not shown).

Table 4 also shows the time interval between DM2 diagnosis and enrolment in the study. Duration of diabetes was not significantly different among the three groups of subjects and, on average, it was approximately 12 years. In addition, Table 4 displays the distribution of antidiabetic treatments in the three study groups. Treatment with metformin was less frequent among HCC patients than among the others; sulphonylureas were the most frequently used drugs among controls, whereas insulin was the most common treatment among HCC and LC patients.

At enrolment, DM2 had been diagnosed for more than 1 year in 86% of study subjects. Based on the records of our Diabetic Clinic, the mean duration of insulin treatment in HCC insulin-treated patients was 5 years; before insulin therapy, patients were treated only with diet. The mean period of treatment with the 2 antidiabetic oral agents was 10 years and before oral antidiabetic therapy patients had only dietary therapy.

Among diabetic subjects, the association of HbA1c levels, antidiabetic therapy, and DM2 duration with the risk of HCC is reported in Table 5. Both when using controls and when using LC as the comparison groups, after adjusting for the potential confounders shown in Table 5, therapy with metformin was associated with a strong and statistically significant reduction (> 80%) of the risk of HCC as compared with the use of sulphonylureas or insulin. In our HCC diabetic patients we found a significant 26%-50% increase in the HCC risk for each 1% increase in HbA1c level. Results did not significantly change in the sensitivity analysis after removing or moving from the diabetic to the non-diabetic category subjects whose diagnosis of diabetes occurred < 12, 24, 60, or 120 mo previously (data not shown).

Table 5 Association of antidiabetic therapy, metabolic control and DM2 duration with HCC among diabetic patients: results of multivariate analyses using controls and LC subjects as comparison groups

	OR[1]	95% CI	P
Control group			
Sex, male	1.860	0.636-5.441	0.2574
Age ≥ 65 yr	0.225	0.071-0.715	0.0115
BMI ≥ 25 kg/m^2	0.888	0.364-2.166	0.7936
Alcohol abuse	16.155	4.798-54.389	< 0.0001
Triglycerides (mg/dL, continuous)	1.001	0.994-1.008	0.8097
Cholesterol (mg/dL, continuous)	1.003	0.994-1.013	0.4890
Metformin *vs* sulphonylureas	0.149	0.039-0.507	0.0054
Insulin *vs* sulphonylureas	1.243	0.459-3.366	0.6686
DM2 duration (mo, continuous)	1.001	0.996-1.005	0.6740
HbA1c % (continuous)	1.265	0.943-1.699	0.1172
LC group			
Sex, male	0.120	0.0051-0.278	< 0.0001
Age ≥ 65 yr	1.508	0.719-3.165	0.2774
BMI ≥ 25 kg/m^2	1.473	0.716-3.029	0.2928
HBV+	3.535	0.428-29.184	0.2410
HCV+	2.870	1.129-7.293	0.0267
Alcohol abuse	1.455	0.567-3.732	0.4351
ALT ≥ 53 IU/L	0.808	0.394-1.675	0.5606
Triglycerides (mg/dL, continuous)	1.002	0.995-1.008	0.6366
Cholesterol (mg/dL, continuous)	1.003	0.995-1.011	0.4012
Metformin *vs* sulphonylureas	0.163	0.057-0.462	0.0006
Insulin *vs* sulphonylureas	0.428	0.203-0.901	0.0255
DM2 duration (mo, continuous)	1.001	0.997-1.005	0.6723
HbA1c % (continuous)	1.508	1.197-1.899	0.0005

[1]Patients with unknown therapy not included in analysis.

DISCUSSION

The results of our study show that chronic poor glycemic control, evaluated by HbA1c testing, is positively associated with risk of HCC in diabetic patients. In addition, therapy with metformin was associated with a strong and statistically significant reduction in the risk of HCC as compared with the use of sulphonylureas and insulin therapy.

Several studies have reported the relationship between DM2 and carcinogenesis in the liver[4-9], but, as far as we know, there are no investigations on the relationship between glycemic control and HCC risk in DM2 patients. In our study, we found that the prevalence of DM2 patients was significantly higher in HCC patients whereas in the LC group it was intermediate between those of HCC cases and controls. After adjusting for potential con-

founders, in our HCC patients DM2 was associated with an increased risk of HCC. The HbA1c mean levels were significantly higher in diabetic HCC patients than in LC and controls but not different between HCC patients with or without HBV and HCV infection or alcohol abuse. In HCC patients (above all in the FU subgroup), the HbA1c values were significantly higher and the mean levels during previous years of diabetic life were similar to those recognized at HCC occurrence. Multivariate analysis showed that high levels of HbA1c among diabetic HCC patients were associated with a significantly increased risk of liver cancer.

Because of IR, patients with DM2 have a long-term exposure to increased circulating insulin levels. It is well known that insulin stimulates cellular mitosis by direct action[14], and indirectly by stimulating the insulin-like growth factor-1 intracellular pathway, a major mitogenic and anti-apoptotic effector in carcinogenesis[15]. Sulphonylurea treatment increases insulin secretion and its circulating levels: this effect is detrimental in terms of weight gain and insulin levels[38]. In contrast, metformin treatment can ameliorate IR and reduce weight gain. The ability of metformin to reduce the insulin plasma levels and to activate cellular AMP-activated protein kinase (AMPK) represents, respectively, its direct and indirect proposed anti-oncogenic mechanisms. In fact, metformin not only lowers blood glucose and insulin levels but, through AMPK activation, also attenuates the *in vitro* response of cancer cells to insulin[39,40].

Several *in vivo* studies showed that cancer risk was lower in patients exposed to metformin than in unexposed patients[41,42]. Metformin has also been shown to be potentially beneficial in patients with specific types of cancer. For example, DM2 patients receiving neoadjuvant chemotherapy for breast cancer as well as metformin were more likely to have a complete remission than patients not receiving metformin[43]. In 2 studies patients receiving metformin seemed to have a lower incidence of prostate and pancreas cancer[44,45]. Furthermore, in the ZODIAC study[46], metformin use was associated with lower cancer mortality when compared to non metformin use.

In diabetic patients, good glycemic control prevents the onset and progression of acute and long-term diabetes-related complications[47]; however, it is presently unknown if poor metabolic control of diabetes could enhance or accelerate liver carcinogenesis in patients with CLD and DM2. Furthermore, it is not known whether an optimized control of diabetes can reduce the risk or delay the development of liver cancer. In the European Prospective Investigation into Cancer Study, a 1% increase in HbA1c levels was associated with a 1.3-fold increase in the risk of colorectal cancer[48]; recently it was reported that poor glycemic control is a predictor of clinically aggressive course for the colorectal cancer[49]. HbA1c reflects average glycemia over 3 mo; in our HCC patients, particularly in FU cases, the mean levels of HbA1c were above the recommended target level of 7%; HbA1c was significantly higher in HCC than in controls and LC patients, in which we found mean levels below 7%. Nonetheless it must be emphasized that the HbA1c levels observed in the three groups of subjects in this study were consistent with those seen in the vast majority of Italian diabetic patients[10].

It is known that diabetes is associated with more advanced lesions and poor outcomes in patient with HCC[50] but the observation that DM2 precedes the diagnosis of HCC in the majority of our cases, suggests that glucose intolerance and glycemic control are an outstanding feature of the hepatic tumor that may not always be related to the liver cancer.

This was a retrospective, hospital-based study drawn from a clinical series and not from the community: therefore the results may not be representative of those of the general population. There is no evidence that successful treatment for glucose intolerance can reduce hepatic carcinogenesis: therefore only a prospective study is required to demonstrate that optimized glycemic control can modify the risk of HCC in DM2 patients with CLD. However, this is the first study on the relationship between different antidiabetic strategies and the presence of HCC. In our diabetic HCC patients, HbA1c test results are available for years before HCC diagnosis, so a reliable assessment of prior glycemic control was possible. Our study was a single center investigation and all patients of the three groups studied were directly diagnosed and followed-up by us; all biochemical parameters were determined in a single laboratory. The study population was large. The controls, even though recruited from inpatients, were representative of the general population of our Region as regards the prevalence of HCV and HBV infection, alcohol consumption and diabetes mellitus.

To distinguish the temporal relationship between exposure and outcomes, due to the complex and reciprocal relationships between DM2, LC and HCC, we conducted a study on a large group of HCC patients comparing them with both a control group with no liver diseases and a group with LC, that represents the majority of patients with the clinical underlying cause of HCC. To have complete and accurate information on the time interval between the onset of DM2 and the diagnosis of HCC, we reviewed all medical documentation kept, from a period of 9 years before the enrollment of the first patient in the study, at our Diabetes Clinic. All our diabetic patients were on monotherapy of antidiabetic drugs and the selection of the antidiabetic therapy was based on the physician's clinical choice. In particular, in HCC and LC groups, diabetic patients treated with different antidiabetic oral agents had similar basic features as regards alcohol consumption, and hepatic and renal function. To make sure that a temporal relationship existed between diabetes and HCC, a sensitivity analysis was conducted excluding or reclassifying patients with recent diabetes diagnosis, but the results did not change.

Our study confirms that DM2 is associated with an increased risk of HCC. It also shows that in diabetic HCC patients metformin treatment is associated with a strong and statistically significant reduction (> 80%) in the risk of HCC compared with the use of sulphonylureas or

insulin. In diabetic patients with CLD, chronic poor glycemic control, evaluated by HbA1c testing, significantly increases the risk of HCC by 26%-50% for each 1% increase in HbA1c level. It has to be emphasized that in diabetic patients with CLD, it is important not only to attain an optimized glycemic control in order to prevent HCC, but also antidiabetic strategies may have an important effect on the relationship between DM2 and solid tumors.

COMMENTS

Background

In recent years, several investigations clearly indicated that type 2 diabetes mellitus (DM2) is a risk factor for hepatocellular carcinoma (HCC). The aims of the present study were to explore this association and to assess the relationships of antidiabetic therapy and glycemic control with the risk of HCC in DM2 patients with chronic liver disease.

Research frontiers

At the present time the relationship between DM2 and solid cancers is under intensive investigation. It remains unknown if DM2 has a direct carcinogenic effect on the liver and on other parts of the human body.

Innovations and breakthroughs

This study found that DM2 precedes HCC in the majority of patients. The cancer risk seems to be increased by chronic poor glycemic control. Metformin treatment is associated with a strong and statistically significant reduction (> 80%) in the risk of HCC compared with the use of sulphonylureas or insulin.

Applications

The study indicated that in diabetic patients with chronic liver disease it is important not only to attain an optimized glycemic control in order to prevent HCC, but also that antidiabetic strategies may have an important effect on the association between DM2 and solid tumors.

Peer review

This is a potentially important paper that continues to evaluate the link between diabetes and the risk of HCC. Its strength is in its numbers despite being from one institution.

REFERENCES

1 **El-Serag HB**, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004; **126**: 460-468

2 **Bosetti C**, Levi F, Boffetta P, Lucchini F, Negri E, La Vecchia C. Trends in mortality from hepatocellular carcinoma in Europe, 1980-2004. *Hepatology* 2008; **48**: 137-145

3 **Davila JA**, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. *Gut* 2005; **54**: 533-539

4 **El-Serag HB**, Richardson PA, Everhart JE. The role of diabetes in hepatocellular carcinoma: a case-control study among United States Veterans. *Am J Gastroenterol* 2001; **96**: 2462-2467

5 **Verlato G**, Zoppini G, Bonora E, Muggeo M. Mortality from site-specific malignancies in type 2 diabetic patients from Verona. *Diabetes Care* 2003; **26**: 1047-1051

6 **Coughlin SS**, Calle EE, Teras LR, Petrelli J, Thun MJ. Diabetes mellitus as a predictor of cancer mortality in a large cohort of US adults. *Am J Epidemiol* 2004; **159**: 1160-1167

7 **Chen CL**, Yang HI, Yang WS, Liu CJ, Chen PJ, You SL, Wang LY, Sun CA, Lu SN, Chen DS, Chen CJ. Metabolic factors and risk of hepatocellular carcinoma by chronic hepatitis B/C infection: a follow-up study in Taiwan. *Gastroenterology* 2008; **135**: 111-121

8 **Inoue M**, Iwasaki M, Otani T, Sasazuki S, Noda M, Tsugane S. Diabetes mellitus and the risk of cancer: results from a large-scale population-based cohort study in Japan. *Arch Intern Med* 2006; **166**: 1871-1877

9 **Lai MS**, Hsieh MS, Chiu YH, Chen TH. Type 2 diabetes and hepatocellular carcinoma: A cohort study in high prevalence area of hepatitis virus infection. *Hepatology* 2006; **43**: 1295-1302

10 **Bonadonna RC**, Cucinotta D, Fedele D, Riccardi G, Tiengo A. The metabolic syndrome is a risk indicator of microvascular and macrovascular complications in diabetes: results from Metascreen, a multicenter diabetes clinic-based survey. *Diabetes Care* 2006; **29**: 2701-2707

11 **Sloan FA**, Bethel MA, Ruiz D Jr, Shea AM, Feinglos MN. The growing burden of diabetes mellitus in the US elderly population. *Arch Intern Med* 2008; **168**: 192-199; discussion 199

12 **Donadon V**, Balbi M, Casarin P, Vario A, Alberti A. Association between hepatocellular carcinoma and type 2 diabetes mellitus in Italy: potential role of insulin. *World J Gastroenterol* 2008; **14**: 5695-5700

13 **Donadon V**, Balbi M, Zanette G. Hyperinsulinemia and risk for hepatocellular carcinoma in patients with chronic liver diseases and Type 2 diabetes mellitus. *Expert Rev Gastroenterol Hepatol* 2009; **3**: 465-467

14 **Calle EE**, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat Rev Cancer* 2004; **4**: 579-591

15 **Giovannucci E**, Michaud D. The role of obesity and related metabolic disturbances in cancers of the colon, prostate, and pancreas. *Gastroenterology* 2007; **132**: 2208-2225

16 **Laville M**, Nazare JA. Diabetes, insulin resistance and sugars. *Obes Rev* 2009; **10** Suppl 1: 24-33

17 **Smith U**, Gale EA. Does diabetes therapy influence the risk of cancer? *Diabetologia* 2009; **52**: 1699-1708

18 **Currie CJ**, Poole CD, Gale EA. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. *Diabetologia* 2009; **52**: 1766-1777

19 **Calle EE**, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 2003; **348**: 1625-1638

20 **Hsu IR**, Kim SP, Kabir M, Bergman RN. Metabolic syndrome, hyperinsulinemia, and cancer. *Am J Clin Nutr* 2007; **86**: s867-s871

21 **Barone BB**, Yeh HC, Snyder CF, Peairs KS, Stein KB, Derr RL, Wolff AC, Brancati FL. Long-term all-cause mortality in cancer patients with preexisting diabetes mellitus: a systematic review and meta-analysis. *JAMA* 2008; **300**: 2754-2764

22 **Tazawa J**, Maeda M, Nakagawa M, Ohbayashi H, Kusano F, Yamane M, Sakai Y, Suzuki K. Diabetes mellitus may be associated with hepatocarcinogenesis in patients with chronic hepatitis C. *Dig Dis Sci* 2002; **47**: 710-715

23 **Veldt BJ**, Chen W, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, de Knegt RJ, Zeuzem S, Manns MP, Hansen BE, Schalm SW, Janssen HL. Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. *Hepatology* 2008; **47**: 1856-1862

24 **Konishi I**, Hiasa Y, Shigematsu S, Hirooka M, Furukawa S, Abe M, Matsuura B, Michitaka K, Horiike N, Onji M. Diabetes pattern on the 75 g oral glucose tolerance test is a risk factor for hepatocellular carcinoma in patients with hepatitis C virus. *Liver Int* 2009; **29**: 1194-1201

25 **Rossi M**, Lipworth L, Maso LD, Talamini R, Montella M, Polesel J, McLaughlin JK, Parpinel M, Franceschi S, Lagiou P, La Vecchia C. Dietary glycemic load and hepatocellular carcinoma with or without chronic hepatitis infection. *Ann Oncol* 2009; **20**: 1736-1740

26 **Takamatsu S**, Noguchi N, Kudoh A, Nakamura N, Kawamura T, Teramoto K, Igari T, Arii S. Influence of risk factors for metabolic syndrome and non-alcoholic fatty liver disease on the progression and prognosis of hepatocellular carcinoma. *Hepatogastroenterology* 2008; **55**: 609-614

27 **Stattin P**, Björ O, Ferrari P, Lukanova A, Lenner P, Lindahl B,

Hallmans G, Kaaks R. Prospective study of hyperglycemia and cancer risk. *Diabetes Care* 2007; **30**: 561-567
28 **Llovet JM**, Beaugrand M. Hepatocellular carcinoma: present status and future prospects. *J Hepatol* 2003; **38** Suppl 1: S136-S149
29 **Castera L**, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. *J Hepatol* 2008; **48**: 835-847
30 **Bellentani S**, Tiribelli C, Saccoccio G, Sodde M, Fratti N, De Martin C, Cristianini G. Prevalence of chronic liver disease in the general population of northern Italy: the Dionysos Study. *Hepatology* 1994; **20**: 1442-1449
31 **De Paschale M**, Biagiotti S, Chianese R. Time variation of viral hepatitis B markers in blood donors. *La Trasfusione Del Sangue* 2001; **46**: 335-340
32 **Pilotto L**, Gaggioli A, Lo Noce C, Dima F, Palmieri L, Uguccioni M, Pede S, Giampaoli S, Vanuzzo D. [Diabetes in Italy: a public health problem] *Ital Heart J Suppl* 2004; **5**: 480-486
33 **Bellentani S**, Miglioli L, Bedogni G, Crocè LS, Tiribelli C. Epidemiology of hepatitis C virus infection. *Minerva Gastroenterol Dietol* 2005; **51**: 15-29
34 Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. National Diabetes Data Group. *Diabetes* 1979; **28**: 1039-1057
35 **Hoelzel W**, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I, Hoshino T, John WG, Kobold U, Little R, Mosca A, Mauri P, Paroni R, Susanto F, Takei I, Thienpont L, Umemoto M, Wiedmeyer HM. IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. *Clin Chem* 2004; **50**: 166-174
36 Feasibility of centralized measurements of glycated hemoglobin in the Diabetes Control and Complications Trial: a multicenter study. The DCCT Research Group. *Clin Chem* 1987; **33**: 2267-2271
37 **Corrao G**, Lepore AR, Torchio P, Valenti M, Galatola G, D'Amicis A, Aricó S, di Orio F. The effect of drinking coffee and smoking cigarettes on the risk of cirrhosis associated with alcohol consumption. A case-control study. Provincial Group for the Study of Chronic Liver Disease. *Eur J Epidemiol* 1994; **10**: 657-664
38 **Giovannucci E**. Nutrition, insulin, insulin-like growth factors and cancer. *Horm Metab Res* 2003; **35**: 694-704
39 **Zakikhani M**, Dowling RJ, Sonenberg N, Pollak MN. The effects of adiponectin and metformin on prostate and colon neoplasia involve activation of AMP-activated protein kinase. *Cancer Prev Res* (Phila Pa) 2008; **1**: 369-375
40 **Dowling RJ**, Zakikhani M, Fantus IG, Pollak M, Sonenberg N. Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. *Cancer Res* 2007; **67**: 10804-10812
41 **Evans JM**, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. *BMJ* 2005; **330**: 1304-1305
42 **Libby G**, Donnelly LA, Donnan PT, Alessi DR, Morris AD, Evans JM. New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. *Diabetes Care* 2009; **32**: 1620-1625
43 **Jiralerspong S**, Palla SL, Giordano SH, Meric-Bernstam F, Liedtke C, Barnett CM, Hsu L, Hung MC, Hortobagyi GN, Gonzalez-Angulo AM. Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. *J Clin Oncol* 2009; **27**: 3297-3302
44 **Wright JL**, Stanford JL. Metformin use and prostate cancer in Caucasian men: results from a population-based case-control study. *Cancer Causes Control* 2009; **20**: 1617-1622
45 **Li D**, Yeung SC, Hassan MM, Konopleva M, Abbruzzese JL. Antidiabetic therapies affect risk of pancreatic cancer. *Gastroenterology* 2009; **137**: 482-488
46 **Landman GW**, Kleefstra N, van Hateren KJ, Groenier KH, Gans RO, Bilo HJ. Metformin associated with lower cancer mortality in type 2 diabetes: ZODIAC-16. *Diabetes Care* 2010; **33**: 322-326
47 Implications of the United Kingdom Prospective Diabetes Study. American Diabetes Association. *Diabetes Care* 1998; **21**: 2180-2184
48 **Khaw KT**, Wareham N, Bingham S, Luben R, Welch A, Day N. Preliminary communication: glycated hemoglobin, diabetes, and incident colorectal cancer in men and women: a prospective analysis from the European prospective investigation into cancer-Norfolk study. *Cancer Epidemiol Biomarkers Prev* 2004; **13**: 915-919
49 **Siddiqui AA**, Spechler SJ, Huerta S, Dredar S, Little BB, Cryer B. Elevated HbA1c is an independent predictor of aggressive clinical behavior in patients with colorectal cancer: a case-control study. *Dig Dis Sci* 2008; **53**: 2486-2494
50 **Amarapurkar DN**, Patel ND, Kamani PM. Impact of diabetes mellitus on outcome of HCC. *Ann Hepatol* 2008; **7**: 148-151

S- Editor Tian L **L- Editor** Cant MR **E- Editor** Ma WH