Antidiabetic therapy and increased risk of hepatocellular carcinoma in chronic liver disease

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CONCLUSION: Our study confirms that type 2 diabetes mellitus is an independent risk factor for HCC and preexists in the majority of HCC patients. Moreover, in male patients with type 2 diabetes mellitus, our data shows a direct association of HCC with insulin and sulphanylureas treatment and an inverse relationship with metformin therapy.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide and the third leading cause of cancer-related deaths[1]. In recent years, a significant increase in HCC incidence and mortality rates has been observed in developed countries, but the causes of this growth are only partially understood. Hepatitis C virus (HCV) epidemics certainly play a role, due to the cohort effect of individuals infected in pre-serological age[2]. Although the main risk factors for HCC are HCV, hepatitis B virus (HBV) and chronic alcohol abuse, at least 25% of HCC cases do not have any known etiology, suggesting that further risk factors could be responsible for the increasing incidence of HCC. Diabetes mellitus has recently been proposed as a risk factor for HCC[3]. During the past two decades, the prevalence of diabetes mellitus, and in particular of type 2 diabetes mellitus, has dramatically increased in many countries, including Italy[4]. Sedentary...
lifestyles, excessive food consumption and obesity appear to be the main causes of the current diabetes mellitus epidemic in western world[8].

Previous studies on the association between diabetes mellitus and liver diseases showed that type 2 diabetes mellitus appears to be a cause of non-alcoholic fatty liver disease (NAFLD) and that cirrhosis and HCV infection increase the susceptibility to diabetes mellitus[6,7].

Moreover, conflicting results were reported on the association between diabetes mellitus and solid tumors, in particular HCC[3,8-12]. While earlier investigations did not report any association between diabetes mellitus and HCC, recent data clearly indicate that diabetes mellitus is a risk factor of HCC[13-15].

However, the precise relation between diabetes mellitus and chronic liver diseases still needs to be further investigated. Therefore, the aims of this study are to explore the association between HCC and diabetes mellitus in a large cohort of patients with HCC and to describe the temporal relationship between the onset of diabetes and the development of HCC. We also considered the clinical and metabolic characteristics of the patients with type 2 diabetes mellitus and HCC, as well their antidiabetic therapy.

MATERIALS AND METHODS

Ethics
This work has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association.

We performed a retrospective, case-control study on three groups of Caucasian individuals, attending the Liver Unit and Diabetic Clinic of 3rd Internal Medicine in the Pordenone General Hospital (Pordenone, Italy) from January 1994 to June 2006. The 3rd Internal Medicine of Pordenone Hospital is a tertiary referral centre for liver disease and diabetes mellitus. This study is a single centre investigation and all patients of the three groups studied were all afferent, directly diagnosed and followed-up in the 3rd Internal Medicine of Pordenone Hospital.

A series of patients with HCC was compared with two different groups: one consisted of patients with liver cirrhosis and the other one, the controls, included individuals who were treated in our Hospital for a wide spectrum of acute conditions.

HCC group
This group comprised 465 consecutive patients with HCC, of which 398 cases (85.6%) were diagnosed by means of cytological or histological examination of hepatic focal lesions. The others (14.4%) were diagnosed according to the following acknowledged criteria[14]: ultrasound examination (also by using micro-bubbles of sulphur hexafluoride as contrast dye in the last three years), α fetoprotein (AFP) > 400 ng/mL, computerized tomography scan and/or magnetic resonance imaging of the upper abdomen. Clinical data, biochemical parameters and the antidiabetic treatment were considered at the time of HCC diagnosis.

Patients with HCC were further divided in two subgroups: the follow-up (FU) and the clinically overt group (CO). The FU group comprised 305 patients with small, single hepatic tumors who received the diagnosis during a surveillance program of HCC in cirrhotic patients based on ultrasound examinations and AFP determinations every 3-6 mo. The clinically overt group (CO) comprised 160 cases with advanced, large size and symptomatic HCC at diagnosis.

LC group
We enrolled 618 patients with liver cirrhosis (LC), matched with HCC cases by age (± 5 years), gender, body mass index (BMI), transaminases, history of diabetes, prevalence of HBV and HCV infections, alcohol consumption and time of admission. These patients were admitted to our Hospital for diagnosis, staging or therapy of liver cirrhosis. Clinical data, biochemical parameters and antidiabetic treatment were considered at the time of recruitment.

According to Child’s classification of cirrhosis, patients were classified as follows: class A: 55.5%; B: 24.3% and C: 20.2%. In the cirrhotic patients, the presence of HCC was ruled out through ultrasound examinations, CT or MRI of the upper abdomen and AFP checks.

Control group
From 28740 in-patients of our region, 490 subjects matched with HCC and LC patients by age (± 5 years), gender, BMI, history of diabetes and time of admission were recruited. Those who were admitted for malignancies, alcohol-related disease, viral liver disease and diabetes mellitus were excluded from our study, although comorbidity of these conditions was not considered as an exclusion criterion. As previously reported[15], the selected control group represented our region’s general population as to HCV and HBV infections, alcohol consumption and diabetes mellitus prevalence in the age group over 65 years.

Methods
The demographic, clinical and biochemical data of each patient were collected in a computerized database. Biochemical parameters were determined at the Pordenone Hospital central laboratory using standardized and validated methods.

Hepatitis B 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 micro particle 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, Pleasanton, CA).

The diagnosis and clinical classification of diabetes mellitus were based on the guidelines of the American Diabetes Association[17,18]. In particular, the distinction
The percentage of type 2 diabetes mellitus patients treated with insulin was similar in the HCC (39.6%) and LC (32.7%) groups, while in only 23 patients (15.1%) diabetes mellitus was diagnosed at least 6 mo before the onset of HCC. We also calculated that the male/female ratio in patients with cryptogenic etiology of HCC was 9.5/1, compared to a male excess of 4.4 in all diabetic patients and of 3.3 in the entire HCC group.

Multivariate analysis
The multivariate analysis in HCC group vs control shows that type 2 diabetes mellitus is associated with an increased risk of HCC occurrence (OR = 2.2; CI 1.2-4.4; P = 0.01), independent of age, gender, BMI, alcohol abuse, HBV and HCV infections.

Time interval from type 2 diabetes mellitus onset to HCC diagnosis
The data collected in the records of our Diabetes Clinic show that in 122 patients (84.9%), type 2 diabetes mellitus was diagnosed at least 6 mo before the onset of HCC, while in only 23 patients (15.1%) diabetes mellitus was recognized after the diagnosis of HCC.

The time interval between diabetes mellitus diagnosis and HCC onset was exactly calculated: diabetes was found to be present prior to the HCC diagnosis for a mean time of 141.5 ± 9.4 mo.

Moreover, in patients with HCC of cryptogenic etiology, diabetes mellitus was present before HCC occurrence for a mean period of 150.5 ± 10.1 mo.

In the subgroup with pre-existing type 2 diabetes mellitus, the time interval until the diagnosis of HCC was longer in insulin treated patients than in those treated with antidiabetic oral agents (171.5 ± 87.6 mo vs 118.7 ± 95.2 mo; P = 0.05).

### RESULTS

Each subject with diabetes mellitus in the HCC and LC groups showed the clinical and metabolic characteristics of type 2 diabetes mellitus. Of note, none of our HCC or liver cirrhosis patients had type 1 diabetes mellitus.

As shown in Table 1, the prevalence of type 2 diabetes mellitus was 31.2% in the HCC group, 23.3% in the LC group and 12.7% in the controls. The prevalences of type 2 diabetes mellitus in the three groups were statistically different, with an OR of 3.12 (CI 2.2-4.4) in HCC group vs controls and an OR of 2.09 (CI 1.5-2.9) in LC vs controls group.

### Table 1

| Subjects | DM2 -ve n (%) | DM2 +ve n (%) | OR (95% CI) | P |
|----------|----------------|---------------|-------------|---|
| Total    | 320 (68.8)     | 145 (31.2)    | 3.12 (2.2-4.4) | < 0.001 |
| HCC (465)|               |               |             |    |
| Controls (490) | 428 (87.3) | 62 (12.7)     | 2.09 (1.5-2.9) | < 0.001 |
| LC (618) | 474 (76.7)     | 144 (23.3)    |             |    |
| Males    | 264 (67.6)     | 128 (32.4)    | 3.14 (2.1-4.6) | < 0.0001 |
| HCC (364)| 246 (67.6)     | 118 (32.4)    |             |    |
| Controls (385) | 334 (86.7) | 51 (13.3)     | 1.99 (1.3-2.9) | 0.000 |
| LC (459) | 341 (75.8)     | 109 (24.2)    |             |    |
| Females  | 74 (73.3)      | 27 (26.7)     | 3.11 (1.3-7.4) | 0.002 |
| HCC (101)|             |               |             |    |
| Controls (105) | 94 (89.5) | 11 (10.5)    | 2.59 (1.2-5.9) | 0.008 |
| LC (168) | 133 (79.2)     | 35 (20.8)     |             |    |

OR: Odds ratio; CI: Confidence interval. *P < 0.001, HCC group vs control group; †P = 0.00001, HCC group vs control group; ‡P < 0.001, LC group vs control group; ‡‡P = 0.0002, LC group vs control group; ‡‡‡P = 0.002, HCC group vs control group; ‡‡‡‡P < 0.008, LC group vs control group.

Etiology of the chronic liver disease, mean age at HCC diagnosis and type 2 diabetes mellitus prevalence in the subgroups are summarized in Table 2.

The highest prevalence (70.3%) of type 2 diabetes mellitus was found in HCC patients with cryptogenic chronic liver disease.

We also calculated that the male/female ratio in patients with cryptogenic etiology of HCC was 9.5/1, compared to a male excess of 4.4 in all diabetic patients and of 3.3 in the entire HCC group.

### Table 2

| Etiology          | HCC n (%) | Age (yr) | Prevalence of DM2 (%) |
|-------------------|-----------|----------|-----------------------|
| HBV               | 20 (4.3)  | 63.3 ± 10.3  | 3 (15.0)               |
| HCV               | 177 (38.1)| 71.5 ± 7.3  | 47 (26.5)              |
| Alcohol           | 141 (30.4)| 66.7 ± 8.5  | 52 (26.9)              |
| HBV + HCV         | 8 (1.7)   | 60.8 ± 12.8 | 2 (25.0)               |
| HBV + alcohol     | 9 (1.9)   | 62.9 ± 9.3  | 2 (22.2)               |
| HCV + alcohol     | 81 (17.4) | 67.7 ± 9.2  | 27 (33.3)              |
| HBV + HCV + alcohol| 2 (0.4)| 68.4 ± 10.3 | 0                     |
| Cryptogenic       | 27 (5.8)  | 68.6 ± 9.3  | 19 (70.3)              |

*P < 0.001, HBV vs HBV + HCV; HCV vs HBV; HCV vs HBV + alcohol; HCV vs HCV + alcohol; HBV vs alcohol; †P = 0.048: HBV vs alcohol; ‡P < 0.001, cryptogenic vs HCV; ‡‡P < 0.002, cryptogenic vs alcohol.

Statistical analysis
Normality tests were performed on all data. Parametric data are expressed as mean ± SD. Data with multiple time points variables were analysed by the general model ANOVA. Post hoc multiple comparisons were performed using an LSD test when ANOVA testing was significant (P ≤ 0.05). To establish univariate associations among variables, the odds ratio (OR) with a confidence interval of 95% was calculated, using the simple analysis of the logistic regression. All statistical analyses were performed using SPSS software 13.0 for Windows (SPSS Inc, Chicago, IL).

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ported that almost all (99%) diabetic patients who developed HCC had type 2 diabetes mellitus, and Lai et al. in a survey in Taiwan, observed that type 2 diabetes mellitus, classified according to age at onset of diabetes mellitus, is associated with HCC.

The results of our study are consistent with the theory of the biological mechanisms underlying the epidemiological association between diabetes mellitus and cancer. In fact, this hypothesis postulates that the diabetes-cancer association is likely to be related to insulin resistance and consequent hyperinsulinemia, which are typical features in the majority of type 2 diabetes mellitus patients. Ten years ago, McKeown-Eyssen and Giovannucci observed that risk factors for cancer and insulin resistance in developed countries are almost the same. To explain this analogy, they suggested that protracted exposure to hyperinsulinemia increases the levels of IGF-1, which plays a pivotal role in carcinogenesis (insulin-cancer hypothesis).

In addition, the predictive value of hyperinsulinemia on total cancer mortality and fatal liver tumor incidence has been demonstrated in non-diabetic subjects by two recent prospective analyses.

Liver cirrhosis is a significant cause of death in type 2 diabetes mellitus patients, being even more relevant than cardiovascular diseases. However, patients with type 2 diabetes mellitus often suffer from liver disease as well, and diabetes is a recognized cause of NAFLD and cryptogenic cirrhosis. In fact, it is well-known that the natural history of NAFLD might progress, over a period of many years, from steatosis to steatohepatitis, cirrhosis and, sometimes, to HCC. On the other hand, 20% of patients with cirrhosis have overt diabetes (hepatogenous diabetes) and 60% have impaired glucose tolerance. Thus, the association between diabetes and cirrhosis is complex and reciprocal.

To evaluate the relations between type 2 diabetes mellitus and HCC, regardless of cirrhosis and other risk factors, we performed a single centre, retrospective case-control study on HCC patients comparing them, not only to a group of Control subjects without liver diseases and diabetes mellitus, but also to a series of cirrhotic patients.

Our study shows that the prevalence of type 2 diabetes mellitus in the LC group is intermediate between those of the HCC and controls, indicating that the underlying liver cirrhosis is not the only cause of diabetes in HCC patients.

The similar prevalence of type 2 diabetes mellitus reported in FU and CO subgroups of HCC patients, suggests that the prevalence of diabetes mellitus is not dependent on the size of the liver tumor.

The evidence that type 2 diabetes mellitus is a risk factor for HCC in our patients is obtainable by univariate and multivariate analyses, which show an OR for HCC of 3.12 and 2.2 respectively, similar to those recently reported in U.S. and Asian populations. Moreover, like the results of a recent Japanese investigation, the patients in our study with cryptogenic HCC have the highest percentage of type 2 diabetes mellitus. Interestingly, most patients with cryptogenic HCC are male, suggesting that this sex prevalence could be related to the effect of

Table 3  Antidiabetic therapy in HCC, cirrhotic patients and in Controls with type 2 diabetes mellitus n (%)

|                | Metformin | Sulfonylureas | Insulin |
|----------------|-----------|---------------|---------|
| Total          | 14 (15.9) | 74 (84.1)     | 57 (39.6) |
| HCC            | 15 (31.3) | 33 (68.8)     | 12 (20.9) |
| Controls       | 58 (70.7) | 24 (29.3)     | 62 (43.0) |

a vs b, P = 0.04; a vs c, P < 0.01; b vs c, P < 0.001; d vs b, P < 0.025; e vs b, P = 0.007.

(Table 3). Based on the records of our Diabetic Clinic, the mean duration of insulin treatment in HCC insulin-treated patients was 83.1 ± 63.4 mo; before insulin therapy the patients were treated only with diet.

Antidiabetic therapy and risk of HCC

Univariate analysis in diabetic HCC patients vs diabetic controls shows that the OR for hepatocarcinoma in subjects taking insulin or sulphonylureas was 2.99 (CI 1.34-6.65, P = 0.007) while the OR dropped to 0.33 (CI 0.1-0.7, P = 0.006) in individuals treated with metformin.

Clinical features of type 2 diabetes mellitus in FU and CO groups with HCC

The FU and CO groups showed a similar prevalence of diabetes (30.2% and 33.1%; P = 0.62, respectively). Mean glycated A1c hemoglobin was higher in the FU group (8.2% ± 2.8% vs 7.1% ± 2.1%), although this difference is not statistically significant (P = 0.08). The mean time interval between type 2 diabetes mellitus diagnosis and HCC onset was greater in CO than in FU patients (167.1 ± 114.3 mo vs 127.8 ± 80.1 mo; P = 0.03). Insulin treatment was more frequent in FU cases with type 2 diabetes mellitus than in diabetic CO patients (48.9% vs 28.3%; P = 0.01, respectively).

DISCUSSION

Our study shows that type 2 diabetes mellitus is an independent risk factor for hepatocarcinoma and precedes the onset of HCC. Moreover, we found that insulin or secretagogues antidiabetic oral agents treatment are associated with an increased risk for HCC, while in metformin treated patients the risk of HCC was reduced.

In this study, every HCC and cirrhotic patient with abnormal glucose tolerance showed clinical and pathological characteristics of type 2 diabetes mellitus. This observation confirms the results of two recent prospective studies on the association between diabetes and HCC. El Seragh et al. in a wide study carried out in the US reported that almost all (99%) diabetic patients who developed HCC had type 2 diabetes mellitus, and Lai et al. in a survey in Taiwan, observed that type 2 diabetes mellitus, classified according to age at onset of diabetes mellitus, is associated with HCC.
of diabetes, because the prevalence of type 2 diabetes mellitus, as shown by the current diabetes estimates based on age and sex in the populations of our region and western countries, is higher in men than in women in the age groups in which HCC develops.

The precise temporal relation between the onset of type 2 diabetes mellitus and diagnosis of HCC is only partially understood. A previous prospective study, conducted in a large cohort of males with and without diabetes mellitus, investigated, for the first time, the temporal relationship between diabetes and HCC, showing a two-fold increase of HCC incidence among patients with diabetes.

Our study showed that type 2 diabetes mellitus was present 141 mo before the diagnosis of HCC. Interestingly enough, we observed that in cryptogenic HCC patients, type 2 diabetes mellitus is the only recognized risk factor that these patients have 150 mo before the diagnosis. As already mentioned, patients with small and single hepatocarcinomas and those with advanced tumors have a similar prevalence of type 2 diabetes mellitus. Associated with this, the higher frequency of insulin treatment in FU cases with type 2 diabetes mellitus than in CO diabetic cases, suggests that type 2 diabetes mellitus is more likely to be a cause rather than merely a consequence of the liver cancer.

Our data demonstrates that exogenous insulin treatment is significantly more frequent in diabetic HCC patients than in Control diabetic cases, while cirrhotic patients are more frequently treated with metformin than HCC and Controls individuals. Moreover, in our study, insulin or sulphonylureas therapy are associated with an increased risk of HCC, conversely metformin therapy is associated with a reduced risk of HCC.

These findings are in agreement with previous studies on the relation between antidiabetic therapy and cancer-related mortality, which showed that diabetic patients treated with insulin or sulphonylureas have a significantly high mortality for cirrhosis and HCC, while treatment with insulin-sensitizer drugs that ameliorate insulin action, such as metformin, might have a protective effect on cancer risk.

The effects of insulin therapy in type 2 diabetes mellitus patients with chronic liver disease could be explained by the mitogenic action of exogenous insulin added to those of endogenous hyperinsulinemia. The sulphonylureas might cause an increase both of endogenous insulin secretion and of its precursors, that seem to have mitogenic effects by themselves. Conversely, metformin treatment can decrease insulin resistance, reducing the consequent hyperinsulinemia and its effects in these patients. Moreover, it is known that patients with type 2 diabetes mellitus treated with insulin frequently have more severe insulin resistance, hyperinsulinemia and diabetic complications. Therefore, insulin treatment might be a marker of long lasting and more severe diabetes.

The results of our study, therefore, might have important implications in the clinical management of diabetes mellitus, particularly in males with type 2 diabetes mellitus and chronic liver diseases, because we found that they are at high-risk for HCC. This observation is of primary relevance to the implementation of prevention policies and to encourage the most adequate and cost-effective programs of surveillance in cirrhotic patients. In fact, metformin treatment might have a protective effect and therefore might be recommended as a first-line therapy in diabetic patients with compensated liver cirrhosis, because its use is not associated with the risk of hypoglycaemia and body weight gain.

Thus, our data suggests that patients with type 2 diabetes and chronic liver disease should first control their diabetes mellitus through diet and changes in lifestyle, to decrease their weight and increase physical activity. Subsequently they should take metformin or other insulin sensitizers, to counteract insulin resistance and consequent hyperinsulinemia.

Our study did have some limitations. It is a retrospective study drawn from a clinical series and not from the community; however, in this case-control survey, matching by age, gender, history of diabetes, BMI and time of hospital admission, we selected a control group and we made sure that it represented the general population of our Region. A potential bias in a case-control study like this is discerning temporal relationships between exposure and outcomes, due to the complex relationships between type 2 diabetes mellitus and cirrhosis. To avoid this error, our study was conducted on a large cohort of HCC patients comparing them with both a control group and with a cohort of cirrhotic patients.

Furthermore, a retrospective study like this must be based upon complete and accurate information on the time interval between type 2 diabetes mellitus onset and the diagnosis of HCC. To achieve this, we reviewed all medical documentation kept at the Diabetes Clinic where all the Regional records are filed from the onset of the disease. Therefore, we could review the detailed clinical history of HCC diabetic patients to calculate exactly the individual time interval from the onset of diabetes to the HCC diagnosis. Prospective studies are required to demonstrate that insulin resistance and hyperinsulinemia are the biological mechanisms that explain the association between type 2 diabetes mellitus and HCC. In conclusion, our survey confirmed that type 2 diabetes mellitus is an independent risk factor for HCC and that it precedes HCC diagnosis. Moreover, in male patients with type 2 diabetes mellitus, our data show a direct association of HCC with insulin and sulphonylureas treatment and an inverse relationship with metformin therapy.

**COMMENTS**

**Background**

Type 2 diabetes mellitus has been associated with hepatocellular carcinoma (HCC). However, the relationship between type 2 diabetes mellitus and the underlying liver cirrhosis, and the effects of antidiabetic therapy on HCC risk have not yet been fully evaluated.

**Innovations and breakthroughs**

This study demonstrates that type 2 diabetes mellitus is an independent risk factor for HCC and pre-exists in the majority of HCC patients. In male HCC
patients with type 2 diabetes mellitus, their data shows a direct association of HCC risk with insulin and sulphonylureas treatment and an inverse relationship with metformin therapy.

**Peer review**
This is an interesting and detailed retrospective single center study reviewing patients with diabetes mellitus and chronic liver disease.

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