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Liver iron excess in patients with hepatocellular carcinoma developed on non-alcoholic steato-hepatitis☆

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See Editorial, pages 249–251

***Background/Aims*: Liver iron deposits are frequent in patients with non-alcoholic steato-hepatitis (NAFLD), but their role is not well defined. To investigate the effect of liver iron excess on the prevalence of hepatocellular carcinoma (HCC) in patients with NASH-related cirrhosis.**

***Methods*: Hepatic iron was measured retrospectively with a semiquantitative method in liver biopsies of 153 patients with NASH-related cirrhosis: 51 with HCC and 102 controls without HCC, matched for age, sex and stage of liver disease. The corrected total iron score (0–60) was the sum of three scores: the hepatocytic iron score (0–36), sinusoidal iron score (0–12), and portal iron score (0–12), multiplied by 3/3, 2/3, or 1/3 depending on the localisation of the iron in the nodules.**

***Results*: Conditional logistic regression analysis showed that iron deposits (corrected total iron score > 0) were more frequent in HCC patients than in controls. The median corrected total iron score was significantly higher in HCC patients than in controls. The liver iron overload was sinusoidal.**

***Conclusions*: Iron deposition in the liver was more frequent in patients with NASH-related cirrhosis with HCC than in HCC-free controls. Liver iron overload may be associated with development of HCC in patients with NASH-related cirrhosis.**

***Keywords*: Hepatocellular carcinoma; HCC; NASH; Iron; Liver; Diabetes-mellitus; Ferritin**

1. Introduction

With the increasing prevalence of obesity, abdominal adiposity, diabetes, and metabolic syndrome in the Western world [1], non-alcoholic fatty liver disease (NAFLD) has became the major cause of chronic liver disease [2]. In most cases, NAFLD is a benign condition as regards liver damage; it rarely progresses to non-alcoholic steato-hepatitis (NASH), cirrhosis, and hepatocellular carcinoma (HCC). The frequency of HCC does not differ between patients with NASH-related cirrhosis and patients with chronic hepatitis C [3–7]. Surrogates of NAFLD, obesity and diabetes [4–6] are associated with an increase risk of HCC and contribute to the risk of

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Abbreviations: HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIS, hepatocytic iron score; SIS, sinusoidal iron score; PIS, portal iron score; TIS, total iron score; cTIS, corrected total iron score; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steato-hepatitis.

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HCC in patients with coexisting viral hepatitis [8]. The identification of epidemiologic, biologic, and genetic features that characterize patients at a higher risk of HCC would help to select candidates who require more intensive monitoring [9]. Moreover, identification of predictive factors could shed light on hepatocarcinogenesis and lead to new prevention strategies in these patients [10].

Various additional risk factors for HCC have been identified in patients infected with hepatitis C virus: male sex, increasing age, etc [11–14]. Moreover, liver iron overload, which is frequent in patients with hepatitis C viral infection, [15–17] may exert a carcinogenetic effect thereby facilitating the development of HCC [18–20]. Oxidative stress, which involves the production of iron catalysed oxyradicals, is generally considered to be the main mechanism underlying the progression of fibrosis to cirrhosis and HCC in genetic hemochromatosis [21,22]. However, iron overload may have a detrimental effect also in some chronic liver diseases irrespective of hemochromatosis [23].

In view of these considerations, we carried out an open case-control study to evaluate iron overload status and other potential HCC risk factors in patients affected by NASH-related cirrhosis.

2. Materials and methods

Informed consent was obtained from each patient included in the study; the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the institution's human research committee.

3. Patients

This study was performed in patients who attended the Cotugno Infective Diseases Hospital, the Liver Unit, Clinical and Experimental Hepatology, Department of Internal Medicine, S.G. Moscati Hospital, Avellino, and the Department of Biomorphological Science, University of Naples Federico II, Italy, between January 1999 and April 2007. Patients with histologically proven NASH-related cirrhosis were consecutively enrolled in the study. Cases were patients with HCC, and controls (2 controls for each case) were patients matched for sex, age($\pm$5 years) and disease stage. Controls enrolled had a liver ultrasound negative for focal lesion and a serum α-fetoprotein level $<$40 ng/ml. In all cases, a non-tumoral liver biopsy (taken at least 4 cm from the HCC) was obtained soon after HCC was diagnosed, using a 16-gauge needle. The HCC was sampled with a 21-gauge needle under ultrasound guidance. Exclusion criteria in both groups were: liver sample less than 0.8 cm long, multifragmented biopsies inconclusive for histological examination and diagnosis; a family history of hemochromatosis or the classic clinical expression of hemochromatosis (skin pigmentation, cardiac failure, diabetes, hypogonadism, and arthritis). Both patients and controls underwent genetic testing for hemochromatosis. HFE C282Y and H63A mutations were tested by the polymerase change reactin on DNA extracted from dried blood spots on filter paper blotters. C282Y or H63Y heterozygotes or homozygotes were excluded from the study. We excluded patients with hemochromatosis in order to minimize heterogeneity in the population. Other exclusion criteria were haemolytic disease, porphyria cutanea tarda, blood transfusions and serological markers for hepatitis B and C virus infection, antinuclear antibody titre $>$1:40; positive assays for anti-smooth muscle antibody, antimitochondrial antibody, abnormal ceruloplasmin and an α_1 antitrypsin phenotype. Alcohol consumption was assessed with a self-administered lifetime questionnaire. We calculated the mean lifetime daily alcohol intake, for past and present, expressed as grams of ethanol per day, for all patients. An intake above 20 g/day for men and women in the past or present was considered an exclusion criteria. The patients' alcohol history was elicited also from the referring doctor and the patients' relatives. We selected this low threshold level of alcohol intake to reduce the possibility of misclassifying non-alcoholic versus alcoholic liver disease. Surrogate biochemical markers of alcohol consumption were not used.

We collected the following data: age, sex, oesophageal varices (small, grade I; large, grades II and III), and degree of impairment of liver function estimated using the Child-Pugh score based on serum albumin, bilirubin, prothrombin time, presence of ascites, and encephalopathy. We also recorded body-mass-index (BMI), systemic-hypertension and diabetes-mellitus, serum alanine-aminotransferase, aspartate-aminotransferase, and γ-glutamyl-transferase. Diabetes-mellitus was diagnosed according to the American Diabetes Association criteria [24]. BMI was calculated by dividing the weight (in kg) of the patient by their height (in m) squared. Hypertension was diagnosed if patients were on antihypertensive drug therapy or if patients had blood pressure higher than 145/95 mm/Hg, on two separate occasions.

4. Pathological data

A single observer, blinded regarding the patients' details, reviewed the histological sections of tumoral and non-tumoral liver samples of all patients. Histological diagnosis of cirrhosis was based on internationally accepted criteria [25]. Diagnosis of HCC was made according to the World Congress of Gastroenterology Working Party criteria, [26] and subsequent modifications [27]. Cirrhosis was attributed to underlying NASH