Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Mediterranean diet and hepatocellular carcinoma

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Background & Aims: Hepatocellular carcinoma (HCC) has a very poor prognosis and any effort to identify additional risk factors, besides those already established, would be important for the prevention of the disease. Data on the role of diet on HCC risk are still controversial.

Methods: We have evaluated the association of adherence to the Mediterranean diet with HCC risk, as well as the interaction of this dietary pattern with chronic hepatitis infection, by combining two case-control studies undertaken in Italy and Greece, including overall 518 cases of HCC and 772 controls. Adherence to the traditional Mediterranean diet was assessed through the Mediterranean diet score (MDS), which ranges between 0 (lowest adherence) and 9 (highest adherence). Odds ratios (OR) for HCC were obtained through multiple logistic regression models, controlling for potentially confounding factors, including chronic infection with hepatitis B/C viruses.

Results: Compared to MDS of 0–3, the ORs for HCC were 0.66 (95% confidence interval (CI), 0.41–1.04) for MDS equal to 4 and 0.51 (95% CI, 0.34–0.75) for MDS $\geqslant 5$, with a significant trend (p <0.001). The detrimental effect of poor adherence to Mediterranean diet on HCC risk was disproportionally high among those chronically infected with hepatitis B and/or C viruses, with a suggestion of super-additive interaction, albeit statistically non-significant.

Conclusions: Closer adherence to the Mediterranean diet appears to be protective against HCC. Our results also point to potential benefits from adhering to a Mediterranean dietary pattern for patients chronically infected with hepatitis viruses.

Keywords: Liver cancer; Case-control study; Risk factors; Dietary habits; Mediterranean diet.

Received 14 June 2013; received in revised form 16 October 2013; accepted 31 October 2013; available online 14 November 2013

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Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; anti-HCV, antibodies against hepatitis C virus; MDS, mediterranean diet score; OR, odds ratio; CI, confidence interval; BMI, body mass index; RERI, excess risk due to interaction; S, synergy index.

Introduction

Hepatocellular carcinoma (HCC) is the most common histological type of primary liver cancer. The predominant role of chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) in the aetiology of HCC is well documented [1]. Several other risk factors for HCC have been identified, including heavy alcohol intake, tobacco smoking, and obesity [2].

With the exception of aflatoxins, data on the role of diet on liver cancer are inconclusive. Some studies have shown a weak inverse association between fruit consumption and liver cancer risk, and a positive one with glycaemic load, but evidence is sparse and inconsistent [3,4].

It has been reported that for certain diseases, notably coronary heart disease, no food, food group, or nutrient has been implicated as causal, but the evidence for a favorable role of the Mediterranean dietary pattern is convincing [5]. Data are much scantier on Mediterranean diet and cancer risk [6–9]. Because HCC is a disease with a very poor prognosis, with a 5-year survival rate of less than 10% [10], any effort to identify additional modifiable causes of liver cancer would be important in order to allow a more effective prevention of the disease.

We have therefore evaluated the association between the Mediterranean dietary pattern and liver cancer by combining two large case-control studies undertaken in Italy and Greece, two countries in which the traditional Mediterranean diet is still prevalent. Age-standardized mortality from primary liver cancer (mainly HCC) is around 3/100,000 population in these countries [11]. Thus, despite being more common than in most other high

income countries, HCC is still a rare disease, with a lifelong cumulative incidence around 1% in the general population.

Materials and methods

Selection of cases and controls

The present data are derived from case-control studies of HCC in two Mediterranean countries.

The first study was conducted between 1999 and 2002, in the province of Pordenone (north-eastern Italy) and in the city of Naples (southern Italy) [12]. Cases were 258 patients under the age of 85 years with incident (newly diagnosed) HCC. Of the HCC cases, 29 cases did not provide a blood sample and 44 did not provide data on dietary habits, thus leaving 185 eligible cases for the present analysis. The cases excluded for these reasons were similar to those considered in the analyses in relation to major socio-demographic characteristics [13]. Histologic or cytologic confirmation was available for 78.2% of HCC cases, while for the remaining cases, the diagnosis was based on ultrasound, tomography, and elevated alpha-fetoprotein levels. Controls were patients <85 years of age admitted for a wide spectrum of acute conditions to the same hospitals as cases. Patients whose hospital admission was due to diseases related to tobacco smoking, alcohol abuse, hepatitis viruses (e.g., hepatitis, cirrhosis, and esophageal varices), or other chronic diseases were excluded from the comparison group. Blood samples were available for 431 of 462 controls; of these, 412 provided comprehensive questionnaire information on dietary habits and were included in the present analyses. Twenty-seven percent were admitted for trauma, 24% for non-traumatic orthopedic diseases, 25% for acute surgical conditions, 13% for eye diseases, and 11% for other miscellaneous illnesses. In this study, overall, there were 3 refusals in the case series (<1%) and 5 in the control one (<1%).

The second study was conducted between 1995 and 1998 in three teaching hospitals in Athens, Greece, and 374 subjects with incident (newly diagnosed) HCC were identified [14]. Forty-one (11%) of the HCC cases were not enrolled for various reasons (mainly refusals and difficulty in coordinating collection of blood samples in the context of standard medical care). For the 333 cases eventually included in the study, confirmation of their HCC diagnosis was based on biopsy (n = 157), elevated alpha-fetoprotein level (n = 159) or echotomography and/or other methods (n = 14); for 3 cases, details concerning diagnostic confirmation were missing. Controls were 360 patients admitted to the same hospitals as the cases, for injuries, or eye, ear, nose or throat conditions. For each case with HCC, we attempted to select 1 control patient from the same hospital, matching for gender and age. Overall there were 25 refusals in the control series (6%), and a properly matching control could not be identified for some HCC cases. Finally, 360 control subjects were enrolled.

Thus, a total of 518 cases of HCC and 772 controls were considered in the present analysis.

Both studies were approved by participating institutional review boards, and, in both the original case-control studies, written informed consent was obtained from each participant in accordance with the Declaration of Helsinki.

Data collection

In both component studies, information on socio-demographic characteristics, anthropometric measures, selected medical conditions, and lifetime tobacco smoking and alcohol drinking was collected. For dietary assessment, interviewer-administered semiquantitative food frequency questionnaires were used.

In the Italian study, the average weekly frequency of consumption of 63 foods or food groups, as well as complex recipes, two years before cancer diagnosis or hospital admission (for controls) was recorded [15,16]. Detailed information collected on history of alcohol drinking, including any change in alcohol beverage intake, allowed to compute maximal lifetime alcohol intake level. This variable, instead of average alcohol intake, was used in this analysis to take into account the strong tendency of HCC cases to diminish or stop alcohol drinking as a consequence of liver disease before cancer onset. Current and former drinkers were therefore combined on the basis of their maximal lifetime alcohol intake.

In the Greek study, cases and controls were asked to indicate the average frequency of consumption of 120 food items or beverage categories per month, per week or per day, over a period of 1 year preceding the recognition of symptoms or signs of the present disease. Concerning alcohol, the usual intake 1 year before enrolment was recorded.

We calculated energy intakes using country-specific food composition databases [17–19].

In both studies, biological samples were obtained from cases and controls and tests were conducted for hepatitis B surface antigen (HBsAg) and antibodies against HCV (anti-HCV) using third-generation assays.

Mediterranean diet

Adherence to the traditional Mediterranean diet was assessed through an *a priori* score, which is a slight variant of the score reported by Trichopoulou and colleagues [20]. The Mediterranean diet score (MDS) is based on 9 dietary components typical of the traditional Mediterranean diet. For each study subject, a value of 0 or 1 was assigned to each component of the score as follows: for components frequently consumed in the traditional Mediterranean diet (i.e., vegetables, legumes, fruit and nuts, cereals, fish and seafood, as well a high ratio of monounsaturated to saturated lipids), participants whose consumption was above or equal to the study-specific and sex-specific median, calculated among controls, were assigned a value of 1, and 0 otherwise; for components less frequently consumed in the traditional Mediterranean diet (dairy, as well as meat and meat products), participants whose consumption was above or equal to the study-specific and sex-specific median among controls were assigned a value of 0, and 1 otherwise. For alcohol, a value of 1 was assigned to moderate drinkers (that is, men who do drink but no more than 2 glasses per day, and women who do drink but no more than 1 glass every other day) and a value of 0 to those with consumption above these values, as well as to nondrinkers.

The MDS was then calculated by summing up the points for each of the nine components. Thus, the score ranged between 0 (lowest adherence) and 9 (highest adherence).

Data analysis

Odds ratios (OR) of HCC (as estimates of the respective incidence rate ratios) and the corresponding 95% confidence intervals (CI) according to the MDS (in categories, as well as for 1 point increment) were estimated through unconditional multiple logistic regression models, with terms for center, age (categorically, <60, 60–64, 65–70, ⩾70 years), sex, education (categorically, <7, 7–11, ⩾12 years), body mass index (BMI, categorically, <25, 25–24.9, ⩾30 kg/m^2), smoking (current *vs.* never plus former smokers), history of diabetes, non-alcohol energy intake (categorically, in study-specific quartiles among controls), and HBsAg and/or anti-HCV positivity. Multiple logistic regression models with the same set of covariates were used to estimate alternately the associations of the individual components of the MDS with HCC. Missing values for adjusting covariates were included as dummy variables in the models.

We estimated ORs for categories of the MDS (i.e., 0–4 as an indicator of poor adherence and ⩾5 as an indicator of close adherence to the Mediterranean diet) and chronic infection with HBV and/or HCV (presence of either or both *vs.* absence of both).

We have evaluated interaction as a deviation from additivity rather than deviation from multiplicatively because the former is more relevant in the biological and clinical context [21,22]. Moreover, multiplicative models typically involve logarithmic transformations and once the logarithm of the number of cases is taken, partitioning of subjects into causal subsets is no more interpretable [21,22]. In order to estimate additive interaction we used the relative excess risk due to interaction (RERI) and the synergy index (S) [22]. RERI is the observed relative risk in the presence of both factors minus the relative risk that would have been expected in the presence of both factors if their effects were exactly additive; it ranges between −infinity and +infinity, with RERI = 0 indicating no interaction (i.e., that the effects of the two exposures are exactly additive), RERI >0 positive interaction and RERI <0 negative interaction. S is the excess risk from both exposures when there is an additive interaction, relative to the risk from both exposures without additive interaction. S ranges from 0 to +infinity; S = 1 means no additive interaction, S >1 positive additive interaction and S <1 negative additive interaction. CI for the indices were obtained using the delta method [23].

Statistical analyses were performed using SAS v.9.1 (SAS Institute, Cary, NC, USA).

Results

Table 1 presents the main characteristics of cases and controls in the Italian and Greek studies, separately, for descriptive purposes. The prevalence of current smokers is higher in the Greek study