Supplementary Information

Influence of Nonalcoholic Fatty Liver Disease with Elevated Liver Enzyme Levels on Risk of Cirrhosis and Hepatocellular Carcinoma

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Study Population and Data Collection

The study participants were healthy individuals aged ≥30 years who participated in a health screening program run by a private health-care institution in Taiwan. All participants were followed from 2008 through 2013. In brief, the participants underwent a series of blood, urine, and anthropometric tests and physical examinations upon study enrolment. Each participant completed a structured questionnaire on sociodemographic characteristics, lifestyle, and personal and family histories of major diseases. Detailed information quantifying alcohol consumption (e.g., amounts, frequencies) was obtained. Blood samples were subjected to virological and biochemical tests such as hepatitis B surface antigen, antibodies against hepatitis C virus, triglycerides, cholesterol, and liver enzymes. Every participant provided signed informed consent for the use of their data generated from medical screenings for biomedical investigations. The study protocol was approved by the Institutional Review Board of the National Yang Ming Chiao Tung University, Taipei, Taiwan.

Follow-Up and Cirrhosis and HCC Diagnoses

The National Health Insurance database was used to identify patients with cirrhosis. The following criteria were used to classify newly diagnosed cirrhosis cases: at least one hospital admission code with cirrhosis diagnosis or with 2 or more outpatient visits. The hospital admission date or the first date of outpatient visit that met the criteria for cirrhosis, whichever came first, was used as the date of incident event. Cirrhosis events were identified according to the International Classification of Diseases, Ninth Revision (ICD-9) codes 571.5 and ICD-10 codes K74.0, K74.1, K74.2, and K74.6. In addition, the incidence of HCC and dates of diagnosis were determined according to ICD-9 code 155 and ICD-10 code C22 (through computerized data linkage with the National Cancer Registry).

Statistical Methods

Baseline profiles of the study participants were compared in terms of the presence of NAFLD and elevated liver enzymes (NAFLD with normal liver enzymes, NAFLD with elevated liver enzymes, and non-NAFLD with normal liver enzymes) by using chi-square tests. The duration (person-years) of follow-up was calculated for each participant as the time from the study entry date until the date of cirrhosis or HCC diagnosis, death, or last computerized data linkage with the national health profiles (i.e., December 31, 2015), whichever came first. Incidences of cirrhosis and HCC were derived by dividing the number of cases by the person-years of follow-up. The cumulative risks of cirrhosis and HCC by population with various NAFLD statuses were estimated using the Kaplan–Meier method, and the statistical significance levels of the differences were examined using log-rank tests. Cox proportional hazards models were used to obtain the crude and adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) for NAFLD for the risk of cirrhosis or HCC. The proportionality assumptions (nonchanging HRs over time) of Cox models were examined, and the assumptions were not violated. The potential confounders associated with NAFLD status for cirrhosis and HCC were age, sex, platelet counts, alpha-fetoprotein levels, and diabetes. We also applied stepwise selections in the multiple Cox regression models and performed Cochran–Armitage trend tests to investigate the risk of cirrhosis or HCC in individuals without NAFLD, with NAFLD and normal liver enzyme levels, and with NAFLD and elevated liver enzyme levels. Statistical significance was defined as a 2-sided P value of <.05. We repeated the analyses restricting to participants with BMI < 25 kg/m2 (nonobese) and nonobese participants without diabetes to examine the influence of NAFLD on the risk of cirrhosis or HCC among these populations. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA)