Research Article

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Incidence of non-alcoholic fatty liver disease in Hong Kong: A population study with paired proton-magnetic resonance spectroscopy

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Background & Aims: Because abdominal ultrasonography cannot reliably quantify hepatic steatosis, accurate data on the incidence of non-alcoholic fatty liver disease (NAFLD) are lacking. We aimed to study the population incidence of NAFLD with state-of-the-art non-invasive tests.

Methods: This was a prospective cohort study. The intrahepatic triglyceride (IHTG) content was measured serially with proton-magnetic resonance spectroscopy in community subjects. Transient elastography was performed to assess liver fibrosis.

Results: 565 subjects (mean age 48 years, 62.7% women) without NAFLD at baseline underwent follow-up assessment after a median interval of 47 months (range 34–60 months). 78 (13.8%) subjects developed incident fatty liver with a mean IHTG content of 8.9% (SD 5.3%). 16 (20.5%) subjects had an IHTG content $\geqslant$11.0% suggestive of moderate to severe steatosis. After excluding 2 men with significant alcohol consumption, the population incidence of NAFLD at 3–5 years was 13.5% (95% CI 10.6–16.3%; 3.4% per year). Only 1 subject with incident NAFLD had high liver stiffness (11.1 kPa) suggestive of advanced fibrosis. Metabolic syndrome at baseline was the strongest predictor of incident fatty liver. Incident central obesity developed in 31.0% of subjects with incident fatty liver and 5.6% of those without (p <0.001). No subject with incident fatty liver had regression of impaired fasting glucose, which occurred in 51.1% of those without incident fatty liver (p = 0.001).

Conclusions: 13.5% of the Hong Kong Chinese adult population develop NAFLD in 3–5 years, but few have severe steatosis or advanced fibrosis. Metabolic syndrome is the most important risk factor of incident NAFLD.

Introduction

Non-alcoholic fatty liver disease (NAFLD) affects 15–40% of the general population and is the most common chronic liver disease worldwide [1,2]. Once thought to be a benign disease, NAFLD and its active form non-alcoholic steatohepatitis (NASH) are now believed to be the leading cause of cryptogenic cirrhosis and cryptogenic hepatocellular carcinoma [3–5]. In the USA, NASH has already become the third leading indication for liver transplantation [6].

The current knowledge on the epidemiology of NAFLD is incomplete. Only a few studies have reported the incidence of NAFLD in the general population [7–11]. However, those previous studies assessed hepatic fat using abdominal ultrasonography. NAFLD can only be diagnosed confidently by ultrasonography when hepatic steatosis exceeds 33% [12]. Ultrasonography is also operator-dependent and suffers from intraobserver and interobserver variability. With these limitations, it is probable that some

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Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; ^{1}H-MRS, proton-magnetic resonance spectroscopy; IHTG, intrahepatic triglyceride content; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

patients with incident fatty liver by ultrasonography may all along have NAFLD. Therefore, it is unreliable to study NAFLD incidence by serial ultrasonography. On the other hand, liver biopsy is an invasive procedure and cannot be performed to screen the general population.

In recent years, proton-magnetic resonance spectroscopy (^{1}H-MRS) has emerged as a reliable and reproducible non-invasive test to quantify hepatic fat [13,14]. When performed serially, ^{1}H-MRS is more sensitive than histological grading in detecting changes in hepatic fat over time [15]. Liver stiffness measurement by transient elastography is another non-invasive test of hepatic fibrosis and has been validated in different liver diseases including NAFLD [16,17]. By combining these state-of-the-art assessments, it is now possible to screen for NAFLD and assess its severity non-invasively in a large number of community subjects.

Furthermore, independent genome-wide association studies and candidate gene studies have identified a genetic variant of the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*, rs738409[G], encoding I148M) as one of the strongest genetic factors associated with NAFLD and its histological severity [18–23]. The impact of the *PNPLA3* polymorphism on incident NAFLD has not been studied.

In this study, we studied the incidence and severity of NAFLD in the general population using ^{1}H-MRS and transient elastography, and determined factors associated with incident NAFLD.

Patients and methods

Patients

This was a follow-up study of a population screening project. From 2008 to 2010, we performed ^{1}H-MRS and transient elastography in 922 subjects in the general Hong Kong adult population [2,24]. The subjects were randomly drawn from the government census database and were invited by mail and phone call. 264 (29%) patients were found to have fatty liver, and 154 of them participated in a lifestyle modification trial [25]. In this follow-up study, we repeated ^{1}H-MRS and transient elastography in subjects without fatty liver at baseline after an interval of 3 to 5 years. The subjects were aged 18 years or above. Those with active malignancy, metallic implants or other contraindications to magnetic resonance imaging, positive hepatitis B surface antigen or antibodies against hepatitis C virus, secondary causes of fatty liver (e.g. consumption of amiodarone and tamoxifen) and decompensated liver disease were excluded. The study was approved by the Clinical Research Ethics Committee of The Chinese University of Hong Kong. All subjects provided informed written consent.

Clinical assessment

At baseline and follow-up, the drug history, alcohol intake, smoking and past medical history were recorded using a standardized questionnaire. Alcohol consumption of 20 g daily (140 g weekly) for men and 10 g daily (70 g weekly) for women was the threshold to defined NAFLD [26]. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Waist circumference was measured at the midway level between the lower rib margin and iliac crest with the tape all around the body in the horizontal position. After overnight fasting for at least 8 h, blood samples were taken for liver biochemistry, glucose, insulin and lipids. Insulin resistance was estimated using the homeostasis model assessment (HOMA-IR), which was calculated as fasting plasma glucose (mmol/L) × insulin (mIU/L)/22.5. *PNPLA3* rs738409 allelic discrimination was performed using a TaqMan® SNP Genotyping Assay (Life Technologies, Grand Island, NY).

Metabolic syndrome was defined according to ethnic-specific criteria by the International Diabetes Federation, which was modified from the National Cholesterol Education Program, Adult Treatment Panel III Guidelines, as the presence of any three of the following: (1) central obesity (waist circumference ⩾90 cm in men and ⩾80 cm in women); (2) triglycerides >1.7 mmol/L; (3) reduced high-density lipoprotein-cholesterol (<1.03 mmol/L in men and <1.29 mmol/L in women); (4) blood pressure ⩾130/85 mmHg; and (5) fasting plasma glucose ⩾5.6 mmol/L; or receiving treatment for the above metabolic problems [27].

Magnetic resonance imaging

^{1}H-MRS was performed to measure the intrahepatic triglyceride (IHTG) content within 8 weeks from the baseline and follow-up clinic visits. The same protocol was followed at both time points. A whole-body 3.0T scanner with a single voxel point-resolved spectroscopy sequence and an echo time of 40 ms and repetition time of 5000 ms was used to evaluate a liver volume of 20 × 15 × 40 mm. A no-water-suppressed spectrum was acquired using 32 signal averages and the data were exported for offline spectral analysis. Water (4.65 ppm) and lipid (1.3 ppm) peak amplitudes were measured to determine liver fat content, which was defined as the relative fat signal amplitude in terms of a percentage of the total signal amplitude (water and fat) and calculated as: fat content = $(I_{fat}/(I_{fat} + I_{water}))\times 100$, where I_{fat} and I_{water} are the peak amplitudes of fat and water, respectively. An IHTG content of 5% was used to distinguish between patients with and without fatty liver [2,28].

Transient elastography

Liver stiffness measurements by transient elastography (Fibroscan, Echosens, Paris, France) were performed during the baseline and follow-up clinic visits according to the instructions and training provided by the manufacturer. All operators had performed at least 200 examinations before this study and were blinded to all clinical data and the diagnosis of the subjects. Measurements were performed on the right lobe of the liver through intercostal spaces with the subject lying in dorsal decubitus with the right arm in maximal abduction. Ten successful acquisitions were performed on each subject. The success rate was calculated as the ratio of the number of successful acquisitions over the total number of acquisitions. The median value represented the liver elastic modulus. Liver stiffness measurements were considered reliable if 10 successful acquisitions were obtained and the interquartile range to median ratio of the 10 acquisitions was <0.3. The cut-off value of 9.6 kPa was used to estimate the number of subjects with advanced fibrosis or cirrhosis. This cut-off value has shown a specificity of 92% in a previous validation study using liver histology as the reference standard [17].

Statistical analysis

Statistical tests were performed using the SPSS Statistics version 20 (IBM, Foster City, CA). Continuous variables were expressed in mean (standard deviation) or median (range) and compared using unpaired *t* test and Mann-Whitney *U* test as appropriate. Categorical variables were compared using the χ^2 test. The correlation between baseline and follow-up IHTG content was tested using the Pearson's test. Binary logistic regression with forward conditional selection was performed to identify independent factors associated with incident fatty liver. Receiver-operating characteristics curve analysis was performed to evaluate the performance of alanine aminotransferase (ALT) in detecting incident fatty liver, and the area under curve was compared using the DeLong test. A 2-sided *p* of <0.05 was taken as statistical significance. The current sample size of 565 subjects would determine the proportion of subjects with incident NAFLD at a 95% confidence level and a confidence interval of 2–4%.

Results

The baseline assessment took place from May 2008 to September 2010. Overall, 658 subjects had an IHTG content below 5.0% and were invited to have follow-up assessment. 49 subjects were lost to contact, 41 refused to participate, and 3 died before follow-up. 565 (85.9%) subjects responded and attended the second assessment from October 2012 to October 2013. The median interval between the 2 visits was 47 months (range 34–60 months). Subjects who attended the follow-up visit had similar baseline characteristics to those who did not. At baseline, the mean age of the cohort was 48 years, and 62.7% were women (Table 1). 253 (44.8%) subjects had hypertension or blood pressure above 130/85 mmHg, and 57 (10.1%) had impaired fasting glucose or type