Research Article

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The association of liver enzymes with biomarkers of subclinical myocardial damage and structural heart disease

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Background & Aims: Patients with non-alcoholic fatty liver disease (NAFLD) are thought to be at increased risk of cardiovascular morbidity and mortality. However, the relationships between NAFLD and subclinical myocardial injury or structural heart disease are unknown.

Methods: We conducted a cross-sectional analysis of 8668 participants from the Atherosclerosis Risk in Communities (ARIC) Study, who showed no clinical evidence of cardiovascular disease. We used levels of liver enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST] and gamma-glutamyl transpeptidase [GGT]), in the context of no history of elevated alcohol consumption as non-invasive surrogates of NAFLD. We used highly sensitive cardiac troponin T (hs-cTnT) and N-terminal pro-Brain natriuretic peptide (NT-proBNP) as biomarkers of myocardial damage and function.

Results: In this population-based study (mean age 63 years, 60% women, 78% white), higher levels of ALT, AST, and GGT, even within the normal range, were significantly and independently associated with detectable (hs-cTnT >3 ng/L) and elevated (hs-cTnT $\geqslant$14 ng/L) concentrations of hs-cTnT. The adjusted odds ratios (95% confidence interval) for elevated liver enzymes (*vs.* normal levels) with elevated hs-cTnT were: 1.65 (1.28–2.14) for ALT, 1.90 (1.36–2.68) for AST, and 1.55 (1.13–2.12) for GGT. Furthermore, there was evidence for inverse associations of ALT and AST with NT-proBNP.

Conclusions: Our results suggest that elevated liver enzyme levels in the absence of elevated alcohol consumption may be associated with subclinical myocardial injury. The inverse association between NT-proBNP and both ALT and AST supports the recently described metabolic role of natriuretic peptides.

Keywords: NAFLD; Subclinical cardiac damage; Natriuretic peptides; Troponin T.
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Abbreviations: NAFLD, non-alcoholic fatty liver disease; ARIC, Atherosclerosis Risk in Communities Study; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; hs-cTnT, highly sensitive cardiac troponin T; NT-proBNP, n-terminal pro-Brain natriuretic peptide; LDL, low density lipoprotein; HDL, high density lipoprotein; hs-CRP, highly sensitive C-reactive protein; eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease.

Introduction

Hepatic steatosis, or fatty liver, is characterized by the excessive accumulation of triglycerides in the liver. In the absence of excessive alcohol consumption, this is termed non-alcoholic fatty liver disease (NAFLD), the most common liver condition in western countries. Strictly speaking, liver biopsy is the gold standard method to diagnose NAFLD, although, because of its limitations, it is not routinely performed in clinical practice [1]. Therefore the majority of studies define NAFLD using surrogate markers of the disease such as levels of liver enzymes together with clinical information such as insulin resistance and low levels of alcohol consumption [2].

Besides obesity, NAFLD is associated with type 2 diabetes, dyslipidemia, and hypertension [3,4]. While NAFLD is known to lead to liver related complications [5–9], the role of NAFLD in the development of cardiovascular disease is controversial [10–13]. In addition to shared risk factors, the presence of ectopic fat in the liver is thought to be an important contributor to systemic inflammatory changes [14,15]. Previous epidemiologic studies of the association between NAFLD and cardiovascular disease have mostly focused on atherosclerotic disease and have been limited by the use of small, highly selected samples (e.g. patients with liver biopsy), or the study of patients with already clinically evident atherosclerotic cardiovascular disease [16].

To our knowledge little evidence exists of the association between NAFLD and myocardial damage. Cardiac troponin T and N-terminal pro-brain natriuretic peptide (NT-proBNP) are biomarkers with established value for the identification of subclinical myocardial damage and structural heart disease, respectively [17]. Cardiac troponin T is widely used in the acute care setting to diagnose myocardial infarction. However, recent studies have shown that minute levels of circulating cardiac troponin T measured using novel (pre-commercial) highly sensitivity assays may reflect chronic subclinical myocardial

injury [18], and improve prediction of cardiovascular morbidity and mortality in subjects with stable coronary artery disease, and in persons without clinically evident cardiovascular disease [19–21].

NT-proBNP, an inert fraction of the prohormone for B-type natriuretic peptide (BNP), is secreted by ventricular myocytes in response to increased wall stress and ventricular filling pressure [22–24]. NT-proBNP is a robust biomarker of subclinical left ventricular dysfunction and heart failure, and is associated with cardiovascular disease and all cause mortality [22,25–30].

The objective of this study was to examine the association between NAFLD and subclinical myocardial injury and structural heart disease. We hypothesized that NAFLD, as assessed by liver enzyme levels among people with low alcohol consumption, would be associated with subclinical myocardial damage as indicated by elevated hs-cTnT, and with subclinical structural heart disease, as indicated by elevated NT-proBNP.

Patients and methods

Study population

The ARIC Study is an ongoing cohort of 15,792 middle-aged adults recruited from four U.S. communities: Forsyth Country, North Carolina; Jackson, Mississippi; suburbs of Minneapolis, Minnesota; and Washington County, Maryland [31]. The first examination of participants took place from 1987 to 1989, with three follow-up visits, occurring approximately three years apart, and a fifth from 2011 to 2013. The fourth visit (1996–1998) was attended by 11,656 participants and is the baseline for the present study because both liver enzymes and cardiac biomarkers data are available from this visit. We excluded participants with race/ethnicity other than black or white, persons with a history of coronary heart disease (defined as history of a physician diagnosed myocardial infarction, evidence of a prior myocardial infarction by electrocardiogram, or self-reported prior coronary reperfusion procedure), heart failure (defined as self-reported treatment for heart failure, hospitalization for heart failure, the Gothenburg stage 3, dyspnea due to cardiac causes and under treatment with digitalis or loop diuretics), elevated alcohol consumption (>20 grams/day), individuals with any liver enzyme >4 SD (n = 127), and persons with missing data. The final sample size was 8668 adults.

All participants signed written informed consent and the institutional review boards at each clinical site approved the study.

Measurements of high sensitive cardiac troponin T (hs-cTnT), N-terminal pro–B-type natriuretic peptide (NT-proBNP) and liver enzymes

hs-cTnT concentrations were measured with a novel pre-commercial highly sensitive assay, Elecsys Troponin T (Roche Diagnostics), on an automated Cobas e411 analyzer. The between-assay coefficients of variation were 2.6% and 6.9% for control materials with mean troponin T concentrations of 2.378 μg/L and 0.029 μg/L, respectively [32].

NT-proBNP was measured by using an electrochemiluminescent immunoassay on an automated Cobas e411 analyzer (Roche Diagnostics) with coefficients of variation ranging from 3.5% to 4.7%.

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) were measured using an Olympus AU400e automated chemistry analyzer according to the manufacturer's protocol. Intra-assay coefficients of variation were 11.1% for ALT, 8.5% for AST, and 9.3% for GGT.

Other measurements

Smoking history, alcohol consumption, and family history of diabetes were assessed during interviews with the participants [31].

Using standardized methods, height, weight, waist circumference, and blood pressure were measured. Fasting blood samples were obtained, and the following assays were performed using standard methods: serum glucose, insulin, cholesterol levels (total, LDL- and HDL-cholesterol), triglycerides, creatinine and C reactive protein [33].

Statistical analyses

We modeled liver enzymes using quartiles and dichotomously (normal or elevated). We defined "elevated" as levels >95th percentile based on the gender-specific distributions of each liver enzyme in a healthy subgroup of the ARIC population (normal weight persons without diabetes). Thus, elevated ALT was defined as >24 U/L among men and >21 U/L among women; elevated AST was defined as >32 U/L for men and >28 U/L for women; and elevated GGT was defined as >56 U/L for men and >50 U/L for women.

We modeled hs-cTnT in 3 different ways. 1) Two categories based on commonly used cut-offs: elevated (⩾14 ng/L) *vs.* normal (<14 ng/L); 2) Two categories based on the limit of measurement using this hs-cTnT assay: measurable (⩾3 ng/L) *vs.* non-measurable (i.e. normal, <3 ng/L); and 3) As a continuous variable, participants with non-measurable hs-cTnT were assigned the value 1.5 ng/L.

We also modeled NT-proBNP in 3 different ways: 1) Two categories based on previously used cut-off: elevated (>400 pg/ml) *vs.* normal (<400 pg/ml); 2) Two categories based on detectability of the assay: detectable (⩾5 pg/ml) *vs.* non-detectable (<5 pg/ml); 3) As a continuous variable, participants with non-detectable NT-proBNP were assigned the value 2.5 pg/ml.

We used logistic regression to model the associations of liver enzymes with elevated hs-cTnT or NT-proBNP with multivariable adjustment for potential confounding factors. We tested for interactions by race, sex, obesity, statin use, and diabetes in Model 3. We also implemented linear regression models with each liver enzyme modeled as restricted cubic splines with 4 knots (percentiles 5, 35, 65 and 95) to characterize the shape of the associations of liver enzymes with hs-cTnT and NT-proBNP. All spline models were truncated at the 1st and 99th percentile of the distributions of liver enzymes.

Results

Overall, the mean age of the participants included in the analyses was 62.6 (SD 5.6), 60% were female and 78% were white.

Compared to participants in the lowest quartile of ALT, those in the upper quartiles were more likely to be men and white, had higher body mass index, were more likely to have insulin resistance and diabetes, had lower HDL and higher triglycerides levels, lower levels of c-reactive protein and were less likely to be current smokers (Table 1). Similarly, those in the upper quartiles of AST were also more likely to be men, white and more likely to have insulin resistance. Compared to those in the lowest quartile of GGT, those in the upper quartiles were more likely to be male, black, had a higher body mass index, higher c-reactive protein, had higher LDL and triglycerides levels, and lower HDL, they were more likely to have hypertension, insulin resistance and diabetes.

The characteristics associated with elevated hs-cTnT and NT-proBNP are shown in Supplementary Tables 2 and 3. The following factors were significantly associated with elevated hs-cTnT: older age, gender men, lower education, obesity, diabetes, and hypertension. The factors associated with elevated NT-proBNP included: older age, gender female, current smoking, higher c-reactive protein and hypertension.

Association of liver enzymes with hs-cTnT

Higher quartiles of ALT, AST, and GGT quartiles were significantly and consistently associated with detectable and elevated hs-cTnT (Fig. 1; Supplementary Table 1). Even after adjusting for traditional cardiovascular risk factors, those in the upper quartile of each of the liver enzymes were significantly associated with the presence of elevated hs-cTnT (Table 2). Further adjusting for NT-proBNP did not change results significantly (Supplementary Table 5). We observed similar results in analyses modeling liver enzymes dichotomously (elevated *vs.* not) (Supplementary Table 4). The continuous relationships between each of the liver