Atherosclerosis 240 (2015) 380–386

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Nonalcoholic fatty liver disease is an independent risk factor for atherosclerosis in young adult men

Kadir Ozturk [a, *], Ahmet Uygun [a], Ahmet Kerem Guler [b], Hakan Demirci [a], Cafer Ozdemir [c], Mehmet Cakir [b], Yusuf Serdar Sakin [a], Turker Turker [d], Sebahattin Sari [e], Seref Demirbas [b], Yıldırım Karslıoğlu [f], Mutlu Saglam [e]

[a] Department of Gastroenterology, Gulhane School of Medicine, Ankara, Turkey
[b] Department of Internal Medicine, Gulhane School of Medicine, Ankara, Turkey
[c] Division of Gastroenterology, Department of Medicine, Harvard Medical School, Boston, USA
[d] Department of Health Public and Epidemiology, Gulhane School of Medicine, Ankara, Turkey
[e] Department of Radiology, Gulhane School of Medicine, Ankara, Turkey
[f] Department of Pathology, Gulhane School of Medicine, Ankara, Turkey

ARTICLE INFO

Article history:
Received 23 January 2015
Received in revised form
6 April 2015
Accepted 7 April 2015
Available online 9 April 2015

Keywords:
Nonalcoholic fatty liver disease
Arterial stiffness
Carotid intima media thickness
Flow mediated dilatation

ABSTRACT

Introduction: The possible cause of accelerated atherosclerosis in NAFLD may be the relationship with the MetS and its components. Our primary goal was to evaluate the relationship between NAFLD and subclinical atherosclerosis in adult male patients between 20 and 40 years of age. Moreover, we aimed to investigate the changes in this association according to the presence or absence of MetS.
Method: Sixty-one male patients with biopsy-proven NAFLD and 41 healthy male volunteers were enrolled. In order to exclude any interference of confounding factors, we studied a specifically selected group with no additional cardiovascular risk. PWV, CIMT and FMD levels were measured in all patients and controls.
Results: The levels of cf-PWV were significantly higher in SS and NASH patients compared to the control group ($P < 0.001$); no significant difference was found between SS and NASH patients ($P > 0.05$). We found significantly decreased FMD levels in patients with SS and NASH compared with control subjects ($P < 0.001$). Subjects with NASH had significantly greater CIMT measurements than the SS and controls ($P = 0.026$, $P < 0.001$, respectively). Although, NAFLD patients with MetS had increased cf-PWV and CIMT and reduced FMD compared to healthy subjects ($P < 0.05$), no significant difference existed between NAFLD with Mets and NAFLD without MetS in terms of cf-PWV, CIMT and FMD ($P > 0.05$)
Conclusion: The present study showed that the presence of NAFLD leads to increased risk of endothelial dysfunction and atherosclerosis in adult male patients, independent of MetS.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a term used to describe the fatty infiltration of hepatocytes in the absence of excessive alcohol consumption and other causes of liver disease. The prevalence of NAFLD in Western countries is approximately 15–30% and increases markedly to 70–90% in patients with obesity and type 2 diabetes. NAFLD represents a spectrum of pathological conditions, encompassing simple steatosis (SS), steatohepatitis (NASH), liver fibrosis, and cirrhosis, with the risk of progression to hepatocellular carcinoma. NAFLD is widely accepted as the hepatic expression of metabolic syndrome (MetS) [1–3].

NAFLD has been linked to an increased cardiovascular disease (CVD) risk and based on rapidly expanding evidence, has been proposed to be an independent risk factor for CVD. The possible cause of accelerated atherosclerosis in NAFLD may be the relationship with the MetS and its components [4]. For example, during obesity visceral adipose tissue secretes adipokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 that contribute to vascular inflammation and insulin resistance [5]. However, a growing body of evidence suggests that NAFLD itself might

* Corresponding author. Department of Gastroenterology, Gulhane School of Medicine, Etlik, Kecioren, Ankara 06010, Turkey.
E-mail address: kadirozturk3041@gmail.com (K. Ozturk).

http://dx.doi.org/10.1016/j.atherosclerosis.2015.04.009
0021-9150/

contribute directly to a higher risk of CVD independently of potential confounding factors such as, diabetes mellitus (DM), hypertension (HT), and obesity [6]. Moreover, subjects with NAFLD have increased risk of CVD mortality, independent of other traditional CVD risk factors [7]. The underlying biological mechanisms linking NAFLD and atherosclerosis have not been fully elucidated. Recent studies suggest that it can be associated with increased hepatic insulin resistance, dyslipidemia, oxidative stress, chronic inflammation and decreased adiponectin concentrations [8–10].

There are a few non-invasive procedures, such as carotid intima media thickness (CIMT) measurement and flow mediated dilatation (FMD), that can detect subclinical atherosclerosis in the general population. Arterial stiffness (AS) is another new non-invasive method which has been described recently. Elevated AS is an indicator of structural and functional changes in the blood vessel wall [11]. Carotid femoral pulse wave velocity (cf-PWV) has been accepted as the gold standard for evaluation of AS and is a strong predictor of future cardiovascular events and all-cause mortality [12,13]. To date, several cross-sectional studies have utilized these methods and reported that NAFLD is associated with arterial stiffness, endothelial dysfunction and increased subclinical atherosclerosis. However, the majority of these studies included individuals with ultrasonographically confirmed NAFLD and CVD risk factors [14].

In present study, our primary goal was to evaluate the relationship between NAFLD and subclinical atherosclerosis and also to demonstrate whether the histological severity of NAFLD is correlated with vascular parameters in adult male patients between 20 and 40 years of age. Moreover, we aimed to investigate the changes in this association according to the presence or absence of MetS. In order to exclude any interference of confounding factors for endothelial dysfunction or atherosclerosis, we studied a specifically selected group of young adult men who had no additional cardiovascular risk factors.

2. Methods

2.1. Study population

In this cross-sectional study, 61 male patients with biopsy-proven NAFLD (NASH:39 and SS:22) were consecutively enrolled. All subjects were recruited from individuals who attended the clinic of the Gastroenterology Department, Gulhane School of Medicine, Ankara, Turkey between August 2013 and December 2014. Inclusion criteria for NAFLD were as follows: (I) age ≤40 and ≥20 years, (II) persistently (at least 6 months) elevated aminotransferases, (III) ultrasonographic presence of bright liver without any other liver or biliary tract disease, (IV) evidence of NAFLD on liver biopsy. Patients with any of the following conditions were excluded: previous cardiovascular disease, morbid obesity (BMI > 40 kg/m^2), DM, HT, total cholesterol ≥250 mg/dl, triglycerides ≥400 mg/dl, chronic renal failure, infections (presence of hepatitis B surface antigen, anti HCV, anti EBV Ig M, and anti CMV IgM antibodies), inflammatory disorders and other known causes of chronic liver diseases (i.e. autoimmune hepatitis, hemochromatosis, primary biliary cirrhosis, Wilson's disease, α1 antitrypsin deficiency). We also excluded alcohol consumption >140 g/week, as well as exposure to occupational hepatotoxins or drugs. The forty-one control subjects (age 20–40 years), recruited from hospital staff members and relatives, consisted of 41 apparently healthy male volunteers with normal liver function tests and liver ultrasonography. The study was approved by the local ethics committee of Gulhane School of Medicine, in accordance with the declaration of Helsinki. Informed consent was obtained from all patients and healthy subjects who met inclusion/exclusion criteria.

2.2. Clinical and laboratory assessments

Clinical data including medical history, smoking status, and drug use were taken from each participant. Body weight and height were measured in subjects with light clothes and without shoes. Body mass index (BMI) was calculated as the ratio of weight (kg)/height^2 (m^2). Overweight was defined as a BMI between 25 and 30 and obesity was defined as BMI > 30. Waist circumference (WC) was measured at the mid-point between the lower ribs and the iliac crests at the end of normal expiration. The hip circumference (HC) was measured at the widest level over the greater trochanters. Waist-to-hip ratio (WHR) was calculated as WC divided by HC. All laboratory tests were studied from venous blood following a 12-h fasting. High sensitive CRP (hs-CRP) was measured by using a nephelometric method. The serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), fasting plasma glucose (FPG), uric acid, high density lipoprotein (HDL), triglycerides (TG) and creatinine were determined using auto-analyzers. LDL was calculated by using the Friedewald's formula [15].

Oral glucose tolerance testing (OGTT) was performed on all participants with the standard 75 g of glucose. Measurements of glucose were performed at 0 and 120 min. Type 2 DM and increased risk of diabetes (prediabetes) were defined according to definition and description of DM by the American Diabetes Association [16]. Blood pressure was measured three times and the last two measures were averaged. HT was defined as documentation of blood pressure higher than 140/90 mmHg. Dyslipidemia was defined as the presence of total cholesterol level ≥260 mg/dl or low density lipoprotein (LDL) level ≥160 mg/dl. The fasting insulin level was measured in duplicate by the chemiluminescence method using reagents from Roche Diagnostics (Mannheim, Germany). Insulin resistance was estimated by modified homeostasis model assessment of insulin resistance (HOMA IR), with the following equation: fasting plasma insulin (mU/ml) × fasting plasma glucose (mg/dl)/405 [17].

Subjects with NAFLD were divided two groups according to the presence of MetS. The modified National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria was used to define MetS and it was defined by the presence of at least three of more of the following criteria: (I) central obesity: WC ≥94 cm in men, ≥80 cm in women, and/or BMI ≥ 25 kg/m^2 in both sexes, (II) TG ≥ 150 mg/dl, (III) HDL-c ≤40 mg/dl in men and ≤50 mg/dl in women; (IV) blood pressure ≥130/85 mm/Hg; (V) FPG ≥100 mg/dl, or previously diagnosed type 2 DM [18].

2.3. Liver histology

Liver biopsy, which is the gold standard to define NAFLD, was performed with ultrasonographic guidance by one gastroenterologist in all patients with NAFLD. All biopsies were at least 2 cm in length and contained a minimum of 8 portal tracts. The slides were stained with hematoxylin and eosin and an experienced hepatopathologist who was blinded to subjects' details scored each liver biopsy specimen using the semiquantitative classification of Kleiner et al. [19]. Briefly, the degree of steatosis, liver injury, and inflammatory activity were scored using an 8-point scale (steatosis 0–3; lobular inflammation 0–3; ballooning hepatocyte degeneration 0–2). Histopathologic features were graded according to the NAFLD activity score (NAS), in which a score of ≥5 was defined as NASH. The stage of fibrosis was graded using a 6-point scale as: 0 = no fibrosis, 1a,b = mild (1a)/moderate (1b) zone 3 and perisinusoidal fibrosis; 1c = portal fibrosis only; 2 = zone 3 and portal/periportal fibrosis; 3 = bridging fibrosis; 4 = cirrhosis.