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Non-alcoholic fatty liver disease fibrosis score and preclinical vascular damage in morbidly obese patients

Alessandra Gentili[a,*], Giulia Daviddi[a], Stefano De Vuono[a], Maria Anastasia Ricci[a], Francesco Di Filippo[a], Abdalkader Alaeddin[a], Massimo R. Mannarino[a], Marcello Boni[b], Gaetano Vaudo[a], Graziana Lupattelli[a]

[a] Internal Medicine, Angiology and Atherosclerosis, Department of Medicine, "Santa Maria della Misericordia" Hospital, University of Perugia, Italy
[b] Department of Surgery, "San Giovanni Battista" Hospital, Foligno (Perugia), Italy

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is strongly related with enhanced morbidity and mortality from cardiovascular disease. In obese patients with both NAFLD and features of the metabolic syndrome, the cardiovascular risk is further increased.
Aim: The aim of this study is to investigate the relationship between severity of liver fibrosis evaluated by NAFLD fibrosis score (NAFLD-FS), Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), other obesity-related markers and preclinical atherosclerosis in morbidly obese patients with previously diagnosed NAFLD.
Methods: Laboratory parameters, visceral fat area (VFA), flow-mediated dilatation (FMD), intima-media thickness (IMT), HOMA-IR and NAFLD-FS were determined in 196 morbidly obese patients.
Results: Patients with higher NAFLD-FS or HOMA-IR show higher left max-IMT and lower FMD ($p < 0.001$). VFA and NAFLD-FS, but not HOMA-IR, were independent predictors of reduced FMD (respectively β −0.268, $p = 0.001$ and β −0.165, $p = 0.039$, p of the model < 0.001) and increased left max-IMT (respectively β 0.165, $p = 0.031$ and β 0.301, $p < 0.001$, p of the model < 0.001).
Conclusions: In morbidly obese patients, NAFLD-FS correlates with markers of early vascular damage. NAFLD-FS, easier to obtain than VFA, seems to be a better score than HOMA-IR to categorize such subjects who are potentially at risk of future cardiovascular events.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) includes a set of pathological conditions ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), which can progress to end-stage liver diseases [1]. NAFLD is strongly associated with all the features of the metabolic syndrome, particularly with obesity: approximately 80% of patients with severe obesity (bariatric surgery candidates) have NAFLD, with high prevalence of the severe forms of the disease [2,3].

There is growing evidence that NAFLD, especially in the advanced forms, is strongly related with enhanced morbidity and mortality from cardiovascular disease [4–7]. Moreover, in patients with both NAFLD and features of the metabolic syndrome, the cardiovascular risk is further increased [8]. This close connection between NAFLD and cardiovascular disease should lead to analyze the relationship between NAFLD and atherosclerosis and to find markers of early vascular damage in patients with steatosis. Indeed, recent studies demonstrated that NAFLD is closely associated with increased intima-media thickness (IMT) [9–11] and reduced brachial artery flow mediated dilatation (FMD) [12], which are approved and reliable markers of subclinical atherosclerosis. However, if NAFLD can contribute per se to the development of early vascular damage or if this relationship is only mediated by the concomitant cardiometabolic risk factors is still a matter of debate. Many studies argue that the association between NAFLD and increased IMT is dependent on the presence of insulin-resistance or features of the metabolic syndrome [13–15]. However, others have found opposite results and the question is still unresolved [16,17].

Liver biopsy represents the "gold standard" test for diagnosis, assessment of severity and prognosis of NAFLD, but is an

* Corresponding author at: Internal Medicine, Angiology and Atherosclerosis, Department of Medicine, "Santa Maria della Misericordia" Hospital, University of Perugia, Piazzale G. Menghini 1, Sant'Andrea delle Fratte, 06129 Perugia, Italy. Tel.: +39 075 5853525; fax: +39 075 5784022.
E-mail address: alessandra.gentili1987@gmail.com (A. Gentili).

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invasive, risky and costly procedure, often with concomitant complications [18,19]. Therefore, it cannot be performed on all patients with NAFLD and considered as a screening tool in daily practice [18]. Hence, various noninvasive scoring systems composed of routinely measured clinical and laboratory variables have been recently proposed to assess the presence of fibrosis and its severity, including NAFLD Fibrosis Score (NAFLD-FS) [20]. To-date this is the most validated noninvasive index used in NAFLD to identify patients with and without advances fibrosis [20–22]. Angulo et al. who introduced NAFLD-FS, established two cut-off points, dividing patients in three groups based on the probability for advanced fibrosis: low probability (NAFLD-FS: <−1.455), intermediate probability (NAFLD-FS: −1.455 to 0.675) and high probability (NAFLD-FS: >0.675). Many studies have found that NAFLD-FS is the best indicator of patients at risk for liver-related complications or death [21,22] but little is known about the possibility of using this score to identify, in the subgroup of patients with NAFLD, those with high risk for cardiovascular disease [23].

Due to the significant association between NAFLD and cardiovascular disease, identifying subjects with NAFLD and detect the presence and the severity of liver fibrosis in these patients is of major importance.

The objective of this study is to investigate the relationship between the severity of liver fibrosis (evaluated by NAFLD-FS), insulin-resistance, obesity related markers and preclinical atherosclerosis in a sample of morbidly obese patients with NAFLD.

2. Materials and methods

2.1. Patients

This is a retrospective study and the patients were included from 2012 to 2015. We examined 196 patients, 66 men and 130 women 44 ± 11 years of age, who were consecutively referred to our Clinic for the treatment of morbid obesity. Patients were candidates for bariatric surgery (mean body mass index $44.6 \pm 7.4\,kg/m^2$). Our eligibility criteria included the presence of steatosis and BMI $\geq 40\,kg/m^2$ or 35–40 kg/m^2 and at least one comorbidity. All the subjects had a daily alcohol consumption <20 g/day and were free of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, other chronic liver diseases, liver and renal insufficiency, heart failure (NYHA II-IV), secondary causes of steatosis, secondary causes of obesity and major psychiatric diseases.

The study protocol was approved by Ethics Committee of our Institution, and a written informed consent was signed by all the subjects. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

2.2. Serum measurements of biochemical markers

Glycaemia, insulinemia, glycated haemoglobin (HbA1c), total cholesterol, triglycerides, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), gamma glutamyl transpeptidase (γ-GT), platelet count and albumin were determined in all patients the day of enrollment. The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was used to estimate insulin-resistance [20] in patients that were not under chronic insulin therapy. NAFLD-FS was calculated according to the formula: NAFLD-FS = −1.675 + 0.0037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × impaired fasting glycaemia or diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio − 0.013 × platelet ($\times 10^9$/L) − 0.66 × albumin g/dL) [20].

2.3. Instrumental analysis

After at least a 6-hour of fasting, steatosis was identified by ultrasound examination and was graded using a semi-quantitative scale. In our sample of obese patients, steatosis was graded as grade 1 if there was a slight and diffuse increase in fine echoes in liver parenchyma, as grade 2 if there was a moderate and diffuse increase in fine echoes and deep attenuation of ultrasound signal with slightly impaired visualization of intra-hepatic vessel borders and diaphragm and finally as grade 3 if there was a marked increase in fine echoes with impaired visibility of diaphragm and posterior right lobe of the liver [24].

Ultrasonography equipped with a 3.5-MHz convex array probe (MyLab 50, Esaote, Italy) was used for the measurement of visceral fat area (VFA) using the following parameters (1) the distance between the internal surface of recto-abdominal muscle and the splenic vein, (2) the distance between the internal surface of recto-abdominal muscle and the posterior wall of abdominal aorta at the umbilicus and (3) the thickness of the fat layer of the posterior perinephric space. VFA was then expressed in cm^2 as follows: [VFA] = −9.008 + 1.191 × [distance between the internal surface of the abdominal muscle and the splenic vein (mm)] + 0.978 [distance between the internal surface of the abdominal muscle and the posterior wall of the aorta at the umbilicus (mm)] + 3.644 × [thickness of the fat layer of the posterior right renal wall (mm)] [25]. All the exams were performed by the same sonographer. Intra-operator precision error, expressed as coefficient of variation (CV), was 2%; the coefficient of repeatability was 5 cm^2.

Brachial artery ultrasonography was used to measure flow-mediated dilatation (FMD), expressed as the percentage difference between baseline vessel diameter and diameter measured after reactive hyperemia [26]. Measurements were obtained on non-dominant arm with the patient in supine position after 10–20 min of rest in a quiet, dark room at a standard temperature of 22 °C. A straight, nonbranching segment of the brachial artery above the antecubital fossa was imaged in the longitudinal plane with the ultrasound probe. A blood pressure cuff was placed 1–2 cm below the antecubital fossa and inflated to suprasystolic pressure. Measurements of basal and posthyperemia diameter were recorded at end diastole, and the average of 3 measurements was used for the analysis.

Carotid arteries were examined by high-resolution B-mode ultrasonography as previously described [27] and carried out also in our previous study [24]. An ultrasound device (ESAOTE Technos MP; ESAOTE S.p.A, Genoa, Italy) equipped with a linear multifrequency 7.5–12 MHz transducer was used. Each subject was examined in the supine position and all measurements were obtained at end-diastole with electrocardiographic triggering. The ultrasound images were analyzed using an image processing workstation (AMS System, Gothemburg, Sweden). On longitudinal two-dimensional ultrasound scanning, the image of the far wall of the common artery at the prebifurcation tract was displayed as two bright white lines separated by a hypoechogenic space. The distance between the leading edge of the first bright on the far wall (lumen–intima interface) and the leading edge of the second bright line (media-adventitia interface) indicates the IMT of the far wall. The maximum values of the intima–media thickness were calculated on both the left and right carotid artery and defined as the left max-IMT and right max-IMT, respectively. All ultrasound studies were performed by the same investigator. The intraobserver coefficient of variation was 1.1%.

Bioimpedentiometry with electrodes applied to the foot plantar surface was used to measure the fat mass as a percentage of body weight (Tanita Body Composition Analyzer TBF-410).

All the instrumental analysis was performed the day of enrollment.