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Subclinical cerebrovascular disease in NAFLD without overt risk factors for atherosclerosis

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ABSTRACT

Background and aims: Non-alcoholic fatty liver disease is recognized not only as part of the metabolic syndrome but also as an independent predictor of cardiovascular disease.
Methods: In this study, NMR spectroscopy method, together with perfusion techniques, was used to detect subclinical brain vascular damage in subjects with NAFLD without overt atherosclerosis risk factors (i.e. hypertension, diabetes, hypercholesterolemia, obesity).
Results: The results suggest that subjects with histologically proven NAFLD have a reduced cerebral perfusion (CBFr) confined to limited brain areas, i.e., left semioval center and posterior cingulate cortex. No statistically significant differences in CBFr values were found, dividing the NAFLD cohort into subgroups, considering NAS score, presence/absence of NASH/fibrosis, and degree of steatosis.
Conclusions: Our data suggest that NAFLD per se may be involved in cerebral atherosclerotic disease. It will be interesting to draw longitudinal studies to determine whether these changes could evolve in more serious cerebral injury.

1. Introduction

Non alcoholic fatty liver disease (NAFLD), a spectrum of diseases ranging from simple steatosis to steatohepatitis (NASH) and cirrhosis [1], is characterized by abnormalities of glucose and lipid metabolism that relate it to the metabolic syndrome (MetS) [2]. NAFLD is often associated with conditions characterized by an increased cardiovascular risk, such as overweight/obesity, dyslipidemia, type 2 diabetes mellitus (T2DM), and hypertension, i.e. the diagnostic criteria for MetS. Nowadays, it is becoming more and more evident that NAFLD itself can raise the risk of developing MetS and the related conditions/disorders beyond well-known predictors, especially with regard to T2DM [3,4].

NAFLD could be not only a marker of MetS but also an independent predictor of cardiovascular disease (CVD) due to its pathogenetic mechanisms, such as abnormal synthesis and secretion of VLDL, insulin resistance (IR), and inflammation. Several studies suggest both IR and hyperglycemia exert independent effects on the development of CVD, leading to a systemic proinflammatory state and an imbalanced lipid metabolism. Clinical data confirm the close relationship between the score of NAFLD activity, revealed by liver biopsy, and markers of vascular damage, above all the carotid intima-media thickness (CIMT) [5,6]. In addition, a tight relation between severity of NAFLD and atherosclerotic coronary artery disease [7,8], and a higher CVD mortality rates in patients with NAFLD have been shown. A comprehensive systematic review underlines the association of NAFLD with subclinical atherosclerosis, i.e., with increased severity of CIMT, coronary calcification, endothelial dysfunction, and arterial stiffness, independently of traditional risk factors and metabolic syndrome [9]. Moreover, NAFLD has been identified as a risk factor for early subclinical abnormalities in myocardial structure and function, such as diastolic left ventricular (LV) dysfunction and mass index [10]. As a result, the early identification of the cardiovascular risk profile in NAFLD becomes a priority, detecting atherosclerosis in young adults before

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they suffer from a major vascular event [11].

Population studies have shown an association between NAFLD and cerebrovascular events in patients with T2DM [12], whereas to date, no clinical studies have investigated the potential link between the extent of liver histological injury in NAFLD and subclinical cerebrovascular damage, independently of classic risk factors. The aim of this study is to investigate the presence of subclinical vascular damage in the brain based on NMR spectroscopy method (MRS), together with perfusion techniques, in subjects with NAFLD in the absence of additional variables classically associated with progression of atherosclerotic disease. To the best of our knowledge, this is the first study examining subclinical cerebrovascular disease in a cluster of highly selected patients with NAFLD free from any other cardiovascular risk factor (CRF). The novelty of the NMR method lies in its ability to detect early cerebrovascular and microstructural brain changes even before the extrahepatic manifestations of the underlying metabolic disease.

2. Patients and methods

Thirty-four subjects with age ranging from 24 to 62 years entered the study. The cohort was divided into two groups of similar age and gender characteristics: NAFLD group (n = 17) and control group (n = 17). The first group encompassed patients followed at the Liver Outpatient Clinic with a clinical diagnosis of NAFLD confirmed by liver biopsy. Members from medical staff and blood donors, whose liver function, lipid panel, and anthropometric measurements were regularly checked, participated as healthy volunteers. Controls did not show steatosis during liver ultrasound examination.

Hepatic ultrasound examinations were performed in all subjects by two experienced sonographers, who were unaware of the participants' clinical and laboratory characteristics at the time of the procedure using a Philips (iU22 Healthcare, Bothell, WA, USA) and Esaote (MyLab70, Italy) device. The degree of hepatic steatosis was defined as absent, mild (1), moderate (2) and severe (3) on the basis of known and accepted criteria (hepatorenal echo contrast, liver brightness, deep attenuation, and vascular blurring).

Exclusion criteria for both groups were the following: hypertension or antihypertensive treatment; T2DM or antidiabetic drugs; active smoking; hypercholesterolemia or statin treatment; cerebrovascular, psychiatric, or neurological diseases; obesity; contraindications to MRI/MRS (e.g. claustrophobia, pacemakers, metal implants).

Anthropometric data (age, sex, BMI) and biochemical data (AST, ALT, γGT, ALP, blood fasting glucose, HbA1c, HOMA-IR index, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides) were recorded for all the subjects with NAFLD participating in the study. As regards the control group, anthropometric data, lipid panel, and liver transaminases were taken into account.

All subjects provided written informed consent according to the ethical guidelines of the 1975 Declaration of Helsinki. The Institutional Review Board “Comitato Etico Milano Area B, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico” reviewed and approved the study project.

2.1. Histologic assessment of liver disease activity

An ultrasound-guided liver biopsy was performed for persistently abnormal liver biochemical tests or increased ferritin in patients with normal aminotransferases. The Kleiner classification was used to grade steatosis, lobular inflammation, and hepatocellular ballooning, and to stage fibrosis from 0 to 4. NASH was considered to be present when steatosis, ballooning, and lobular inflammation were all present. In addition we considered the NAFLD activity score (NAS) graded from 0 to 8, on a scale including separate scores for steatosis, lobular inflammation, and hepatocellular ballooning, and we used the NAS $\geq$ 5 as surrogate of NASH as used in clinical trials [13].

2.2. MRI protocol

All MRI imaging was performed on a 1.5T Siemens Avanto System (Erlangen, Germany) with a 12 channel radiofrequency head coil.

MRI 3D T1 (magnetization-prepared rapid gradient echo [MPRAGE]) and axial T2/proton density-weighted images were acquired and assessed under blinded conditions. Single-voxel ^{1}H-MRS spectra both with and without water suppression were obtained from volumes of interest (8 ml) centered on the visual cortex, hypothalamus, and posterior cingulus. Both non-water-suppressed and water-suppressed spectra were acquired under baseline conditions. A point-resolved spectroscopy (PRESS) sequence was used. The unsuppressed average water spectral linewidth (at FWHM) was 4.4 Hz (±0.2). The water-suppressed acquisition parameters were TR = 4000 ms to minimize saturation effects; TE = 30 ms, and 32 acquisitions. A fully relaxed water signal was also acquired (TR = 10000) to exclude saturation effects. NAA was analyzed as a neuronal marker. Peak areas were obtained by the LC model frequency domain fitting method. Spectra were analyzed without knowledge as to the origin of the spectra.

2.3. Pulsed arterial spin labeling (PASL)

Scanning parameters were TR = 2500 ms, TE = 13.0 ms, TI1 = 700 ms, TI2 = 1800 ms, flip angle = 90°, 100 mm tag with a 10 mm gap between the tag and the imaging slice, FOV = 256 mm, 4.0 × 4.0 × 8.0 mm voxels. A total of 140 PASL-MRI frames (or 70 PASL-MRI pairs) were analyzed for each subject. One high-resolution TI-weighted magnetization-prepared rapid gradient echo (MPRAGE) [TR = 2400.0 ms, TE = 3.23 ms, TI = 1000.0 ms, flip angle = 8°, 1 × 1 × 1 mm voxels] structural scan was also obtained for anatomical region definition and facilitate image alignment.

2.4. Image analysis

Image datasets were transferred to a separate workstation and cerebral blood flow maps for both perfusion techniques were generated using a commercially available software package (Syngo version B17, Siemens, Erlangen, Germany). Separate regions of interest (ROIs) covering hypothalamus, semioval centers, anterior cingulate cortex, posterior cingulate cortex, visual cortex, sensory and motor cortex, and basal ganglia were manually drawn by a single investigator, blinded to patient data.

2.5. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics software (version 23.0). Data are expressed as mean and SD for continuous variables. The two groups of participants (NAFLD patients vs healthy subjects) were compared by independent sample t-tests.

Differences in cerebral regional blood flow among NAFLD subjects with different BMI, degree of steatosis, NAS score, and presence/absence of NASH were assessed using one-way ANOVAs. Moreover, the association of regional cerebral blood flows (CBFrs) with NAFLD was tested by univariate analysis, adjusting for the single variables associated with MetS (triglycerides, HDL cholesterol, blood pressure, and BMI). For all tests, a p value $\leq$ 0.05 was considered statistically significant.