Research Article

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Risk of nonalcoholic steatohepatitis and fibrosis in patients with nonalcoholic fatty liver disease and low visceral adiposity

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Background & Aims: Increased visceral adiposity is considered the hallmark of the metabolic syndrome, whose hepatic manifestation is nonalcoholic fatty liver disease (NAFLD), although a subset of patients does not have visceral obesity. Our study aimed to compare metabolic alterations and liver damage in patients with NAFLD with and without visceral obesity.

Methods: Four hundred and thirty one consecutive patients with liver biopsy-confirmed NAFLD were divided in three groups according to waist circumference, the simplest surrogate marker of visceral obesity. One hundred and thirty three patients (31%) had a waist circumference ⩽94 (males) and ⩽80 cm (females) (group A), 157 (36%) between 94 and 102, and 80 and 88 (B), and the remaining 141 (33%) had values higher than 102 and 88 cm (C).

Results: Significant trends for older age, higher prevalence of female gender, lower HDL, higher triglycerides, altered glucose metabolism, hypertension, and metabolic syndrome were observed with increasing visceral adiposity. In contrast, non-alcoholic steatohepatitis (NASH) detected in 55% and 72% of patients with normal and increased waist circumference, respectively, and the presence of fibrosis ⩾2 were not associated with visceral adiposity. Alanine aminotransferase (ALT), ferritin, HOMA-IR >4, and severe steatosis were independently associated with NASH, whereas ferritin and impaired glucose tolerance were associated with fibrosis ⩾2.

Conclusions: Patients with normal waist circumference, despite milder metabolic alterations, may have NASH and are at risk of developing fibrosis, suggesting that once NAFLD is present, visceral obesity is not a major determinant of liver damage severity.

Keywords: NASH; Waist circumference; NAFLD; Abdominal obesity; Glucose metabolism.

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Abbreviations: NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; HOMA-IR, homeostatic model assessment insulin resistance index; BMI, body mass index; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; OGTT, oral glucose tolerance test; OR, odd ratio; adj, adjusted; CI, confidence intervals.

Introduction

Increased visceral adiposity is considered the hallmark of the metabolic syndrome, a clinical condition characterized by increased cardiovascular risk driven by raised blood pressure, dyslipidemia, and altered glucose regulation. However, in recent years a subset of patients has been identified, with normal body weight and similar metabolic disturbances (so called metabolically-obese, normal weight (MONW) cases), who also shares a similar cardiovascular risk [1].

The various clinical expressions of nonalcoholic fatty liver disease (NAFLD), from pure fatty liver to nonalcoholic steatohepatitis (NASH), cryptogenic cirrhosis, and eventually hepatocellular carcinoma are considered a manifestation of the metabolic syndrome. Also among NAFLD cases, a subset of patients does not present with visceral obesity, but insulin resistance remains the common soil of both the metabolic syndrome and NAFLD, largely independent of increased fat mass. In a prospective study, subjects with NAFLD and elevated ALT were reported to be at higher risk of developing diabetes and the metabolic syndrome than subjects without NAFLD, and the risk was driven by waist circumference, hypertension, and insulin resistance [2].

Conflicting evidence has been reported on the complex relationship between visceral fat mass, insulin resistance and NAFLD. The severity of insulin resistance is a determinant of liver damage progression in NAFLD [3] and adipose tissue insulin resistance was recently proposed to underlie the pathogenesis of liver damage [4–6]. Accordingly, visceral obesity might represent a non-invasive marker of disease severity in the general NAFLD population. However, adequately powered studies assessing the association between waist circumference and liver damage are not available.

Waist circumference remains the simplest and most widely used surrogate marker of visceral adiposity [7,8], and other proposed surrogate markers, including the recent perihepatic adipose tissue thickness at ultrasonography, skin thickness, and dorsal cervical fat are scarcely used in the clinical setting [9,10]. The aim of this study was to compare metabolic alterations and severity of liver damage in patients with NAFLD with and without increased visceral obesity, simply estimated by the easily available waist circumference measure, to define which are the variables associated with hepatic and extrahepatic morbidities.

Methods

Patients

We merged the databases of consecutive patients with liver biopsy-confirmed NAFLD observed in four Liver Units. All consecutive patients who underwent liver biopsy between January 2003 and June 2009 were included in the study unless the tissue sample size was <1.7 cm. The final cohort was made up of 431 cases. Most of these patients had been included in a previous multicenter Italian study [11]. Other causes of liver diseases (viral, autoimmune, cholestatic, drug-induced, hereditary hemochromatosis, Wilson's disease) were excluded. In all patients, daily alcohol intake was lower than 20 g (confirmed by at least one family member). Clinical and laboratory data were collected at the time of liver biopsy (Table 1).

Patients with clinical or imaging evidence of decompensated cirrhosis were excluded from the study. Liver biopsy was performed in 323 (75%) patients because of abnormalities in liver function tests, whereas in the remaining 108 either a persistent increase in serum ferritin or a long-lasting history of steatosis was the main reason for biopsy.

All patients had given informed, written consent to data handling according to a protocol approved by the Senior Staff Committee of our Institutions, a board comparable to an Institutional Review Board.

Methods

The database contained data on life habits, clinical and pharmacological history, BMI, waist circumference (measured in a standing position at the level of the umbilicus) and arterial blood pressure (defined as the mean of the second and third reading of three consecutive blood pressure measurements), blood count, liver function tests (AST, ALT, gamma-glutamyltransferase (GGT), serum albumin, platelets count, bilirubin), fasting glucose and insulin, total and HDL cholesterol, triglycerides, and uric acid. All tests were determined by standard laboratory procedures; insulin by a commercially purchased radioimmunoassay (RIA, Biochem Immunosystems, Bologna, Italy). The upper normal limit of ALT levels was set at 40 U/L. The diagnosis of the metabolic syndrome was carried out according to ATPIII criteria [12], and based on the presence of 3 or more of the following criteria: (1) fasting glucose $\geqslant$100 mg/dl, (2) central obesity (waist circumference >102 cm (men) and >88 cm (women), (3) arterial pressure $\geqslant$130/85 mmHg or treatment for hypertension, (4) triglycerides levels $\geqslant$150 mg/dl or use of fibrates, (5) HDL-cholesterol <40 mg/dl (men) and <50 mg/dl (women).

Insulin resistance was evaluated according to the homeostatic model assessment insulin resistance index (HOMA-IR) [13], as fasting serum insulin (in μIU/ml) multiplied by fasting serum glucose (in mMol/L), divided by 22.5. Oral glucose tolerance test (OGTT) was performed with 75 g of glucose according to World Health Organization criteria in 382 (89%) patients.

The presence of diabetes mellitus (fasting glucose $\geqslant$126 mg/dl, 120-min glucose $\geqslant$200 during OGTT, or treatment with antidiabetic drugs), obesity (BMI >30 kg/m^2) and overweight (BMI, 25–29.9 kg/m^2) were defined using standard criteria.

Liver histology

Liver biopsies were routinely processed and read by a single pathologist in each center. To control for biopsy size, the length of the biopsy was measured with a hand ruler, and the number of portal areas on a cross-section was counted. The minimum biopsy size was 1.7 cm and the number of portal areas 10. The diagnosis of NASH was based on the pathologist's overall impression according to Brunt criteria [14], modified by Kleiner [15]. The stage of fibrosis was scored based on the 5-point scale (stage 0, absence of fibrosis; stage 1, perisinusoidal or portal fibrosis; stage 2, perisinusoidal and portal/periportal fibrosis; stage 3, septal or bridging fibrosis; stage, 4 cirrhosis). The severity of steatosis was graded from 1 to 3 according to the percentage of cells with fatty droplets (1: 6–33%, 2: 33–66%, and 3 >66%).

Statistical analysis

Results are expressed as means ± standard deviations for continuous variables and as frequencies for categorical variables. *p*-Values from ANOVA or chi-square test were considered statistically significant if $\leqslant$0.05. Logistic regression analyses were performed to calculate odds ratios (OR), and their 95% confidence intervals (CI) for two outcomes, NASH, and fibrosis grade $\geqslant$2 (the latter value was chosen because of the relatively mild fibrosis detected in our series) [11]. Variables significant at univariate analyses or of *a priori* interest were entered into the final multivariate model. Independent variables were categorized according to quartiles (ALT), tertiles (ferritin), or following commonly used cut-offs. In particular, waist circumference was categorized in three groups, (group A <94 and <80 cm, group B $\geqslant$94 <102 and $\geqslant$80 <88, group C $\geqslant$102 and $\geqslant$88 cm in man and woman, respectively) [12,16]; steatosis was graded from 1 to 3 [11]. Age was divided in decades, and HOMA-IR categories were arbitrarily chosen as follows: <2.5, absence of insulin resistance; 2.5–4.0, moderate insulin resistance; >4, severe insulin resistance. *p*-Values for linear trends were calculated for ordinal variables. All statistical analyses were performed with the Stata 11 software [17].

Results

The characteristics of patients subdivided in three groups according to waist circumference are shown in Table 1. At increasing waist circumference, patients were progressively older, and more frequently of female gender, had a higher BMI, lower HDL-cholesterol, higher triglycerides, and higher fasting glucose, insulin, and HOMA-IR. In addition, there was an increased prevalence of impaired glucose tolerance and diabetes, arterial hypertension, and metabolic syndrome, which paralleled the increase in visceral obesity.

Table 1. Characteristics of patients with NAFLD, divided according to abdominal circumference (group A<94 and <80 cm, group B$\geqslant$94 <102 and $\geqslant$80 <88, group C$\geqslant$102 and $\geqslant$88 cm in man and woman, respectively).

Variables	Group A (n = 133)	Group B (n = 157)	Group C (n = 141)	*p* value
M/F	128/5	140/17	92/49	<0.001
Age (yrs)	39 ± 10	42 ± 11	47 ± 11	<0.001
BMI (Kg/m^2)	25 ± 2.2	27.5 ± 2.6	30.1 ± 4.1	<0.001
Total cholesterol (mg/dl)	200 ± 39	206 ± 47	207 ± 44	0.29
HDL (mg/dl)	48 ± 13	47 ± 11	45 ± 12	0.03
Triglycerides (mg/dl)	131 ± 86	152 ± 83	164 ± 84	<0.001
Fasting glucose (mg/dl)	93 ± 15	95 ± 24	105 ± 37	0.04
Fasting insulinemia (μU/ml)	14.8 ± 9.9	17.7 ± 12.7	20.8 ± 12.3	<0.001
HOMA-IR	3.4 ± 2.4	4.3 ± 5.0	5.1 ± 3.7	<0.001
ALT (U/L)	71 ± 45	80 ± 47	75 ± 47	0.2
GGT (U/L)	89 ± 104	82 ± 89	88 ± 95	0.46
Serum ferritin (ng/ml)	298 ± 250	339 ± 316	390 ± 337	0.25
Transferrin saturation (%)	35 ± 11	36 ± 12	34 ± 16	0.5
Impaired glucose tolerance	13 (9.8)	15 (9.6)	37 (26.2)	<0.001
Diabetes	7 (5.3)	11 (7.0)	22 (15.6)	0.006
Hypertension	26 (20.6)	52 (34.2)	72 (52.2)	<0.001
Metabolic syndrome	10 (7.5)	25 (15.9)	76 (53.9)	<0.001
NASH	73 (54.9)	83 (52.9)	101 (71.6)	0.002
Fibrosis stage (>2)	37 (27.8)	41 (26.1)	52 (36.9)	0.10
Steatosis grade 3	7 (5.3)	27 (17.2)	36 (25.9)	<0.001

Mean ± SD or number of cases and (%). *p*-Values from ANOVA or chi-square test.