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Association of non-alcoholic fatty liver disease and cardiometabolic risk factors with early atherosclerosis in an adult population in Southern Italy

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Abstract

Aim. The prevalence of risk factors for cardiovascular and metabolic diseases was investigated in an adult population of the city of Cittanova, Southern Italy.

Methods. The study was conducted among 992 randomly selected adults aged 18-75 years, between April 2009 and January 2011.

Results. Prevalence rates of non-alcoholic fatty liver disease (NAFLD), overweight, obesity, and metabolic syndrome (MS) were 24.8%, 41.5%, 27.1%, and 34.4%, respectively. For the components of MS, prevalence of central obesity was 47.4%, impaired fasting glucose (IFG) 34.7%; hypertension 53.7%, low high-density lipoprotein (HDL) cholesterol 34.2%, and hypertriglyceridemia 27.2%.

Conclusions. Hypertension, central obesity, IFG, low HDL cholesterol, hypertriglyceridemia, MS, and increased carotid artery intima-media thickness (IMT) were significantly associated with NAFLD after adjustment for age and sex. With additional adjustment for body mass index (BMI), IMT and MS (depending on the prevalence ratio that was investigated), the positive association between the NAFLD and increased IMT lost statistical significance, while that with body mass index (BMI) and MS remained significant.

Key words

- obesity
- NAFLD
- metabolic syndrome
- cardiovascular risk factors
- IMT

INTRODUCION

The prevalence of overweight and obesity has reached epidemic proportions, and is associated with many diseases and with increased all-cause mortality. Obesity is associated with non-alcoholic fatty liver disease (NAFLD) [1] and with atherosclerosis and its cardiac sequelae, such as coronary artery disease and myocardial infarction [2]. Different measures are used to describe body weight and its abnormalities; body-mass index (BMI) is the most commonly used. Waist circumference and waist-to-hip ratio are likely better indicators of visceral fat accumulation, but are of much less widespread use.

NAFLD is characterized by an excessive accumulation of fat in the liver parenchyma of patients who have no history of alcohol abuse and it is now widely accepted as the most common cause of chronic liver disease in Western countries. The prevalence in the general population has been estimated around 20-30% [3]. NAFLD ranges from simple steatosis to nonalcoholic steatohepatitis and cirrhosis [4]. Increasing severity is accompanied by decreasing reversibility of the disease

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condition. NAFLD is strongly associated with obesity, type 2 diabetes, dyslipidemia, atherosclerosis, and is a risk factor for cardiovascular disease, both in adults and in adolescents [5-7].

To study atherosclerosis, carotid intima-media thickness (IMT) is commonly used as noninvasive marker of atherosclerosis. Increased IMT is associated with the presence and severity of coronary atherosclerosis and cardiovascular disease [11]. In patients with NAFLD, IMT was found to be greatest in patients with nonalcoholic steatohepatitis, intermediate in those with simple steatosis, and lowest in healthy controls matched for age, sex, and BMI [8].

In this population-based study we have investigated the prevalence of NAFLD, cardiovascular and metabolic risk factors, and their association with atherosclerosis in a small city in Southern Italy.

MATERIALS AND METHODS

Setting and target population

The study was conducted from April 2009 to January 2011 by the Associazione Calabrese di Epatologia/ACE (Hepatology Association of Calabria), in collaboration with the Istituto Superiore di Sanità (Italian National Institute of Health). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) and was approved by the ethics committees of participating institutions.

This study was conducted among randomly selected adults in the city of Cittanova (population ~10 376), an inland town in the Calabria Region of Southern Italy. Earnings of most inhabitants of Cittanova are in the middle of the income range. Sex and age distributions are similar to those of Southern Italy in general. A systematic random sample of 1401 (1 every 7) subjects, aged 18-75 years, was selected from the census list updated in December 2008. Of the 1401, 992 (70.8%) agreed to participate. Of the 992 participants 51.7% were females. Written informed consent was obtained from all participants. Biochemical markers and NAFLD were investigated in all study subjects and carotid IMT was measured in a sub-sample, using a systematic random procedure (1 in 2.5).

Physical examination

Weight and height were measured without shoes and with light clothing, and BMI was calculated as body weight (kg) divided by the square of height (m^2). Waist circumference was measured, during expiration, at the narrowest point between the lower rib and the iliac crest. Blood pressure was measured 3 times, after the subject had been seated for at least 10 minutes. The last 2 measurements were averaged for analysis.

Biochemistry and threshold values

Blood samples were collected in the morning after an overnight fast. All blood tests were performed in the same laboratory within a few hours of sampling. Fasting levels of glucose, triglycerides, total cholesterol and high density lipoprotein cholesterol were measured using a Vitros 950 automatic analyzer (Ortho Clinical Diagnostics, Raritan, New Jersey). Low density lipoprotein cholesterol was calculated using the formula of Friedewald.

Values used to define the different conditions were as follows: overweight: BMI 25-29.9 kg/m^2; obesity, ≥ 30 kg/m^2; central obesity: waist circumference ≥ 102 cm in men and ≥ 88 cm in women; impaired fasting glucose (IFG): fasting glucose ≥ 100 mg/dL, ≤ 125 mg/dL; diabetes: fasting glucose ≥ 126 mg/dL or previous diagnosis and/or hypoglycemic therapy; hypertension: systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or previous diagnosis and/or antihypertensive therapy; hypertriglyceridemia: fasting triglycerides ≥ 150 mg/dL, or ongoing therapy; low HDL cholesterol: < 40 mg/dL in men and < 50 mg/dL in women or appropriate therapy; MS: presence of at least three of the aforementioned components.

Ultrasonographic studies

NAFLD was defined as ultrasonographically assessed fatty liver in individuals with alcohol consumption < 140 g/week for women and < 210 g/week for men over a 2-year period prior to the study participation, without other causes of chronic liver disease and not assuming potentially steatogenic drugs.

Liver ultrasonography was performed on all participants by a single sonographer according to a standard protocol previously described [5]. All ultrasound examinations were recorded. The sonographer, blinded to the weight status of the participants, was asked to reexamine 100 recorded, randomly selected ultrasonograms after 1 month. All initial NAFLD diagnoses were confirmed.

Carotid artery ultrasonogaphy was performed, using a 7.5 MHz probe by a single operator and was recorded online [9]. Examination of IMT measurements was performed by an operator blinded to the weight and clinical status of participants.

Statistical analysis

For statistical analysis, population characteristics were reported as proportions, medians and ranges for categorical and continuous variables, respectively. Statistical differences in proportions were evaluated using a c^2 test; a P value < 0.05 defined significance. As NAFLD was highly prevalent, prevalence ratios (PR) were calculated using a regression model with robust variance and constant time at risk [10]. Analyses were carried out using the STATA/IC 13.1 statistical package.

RESULTS

Of the 992 subjects that agreed to participate, 125 subjects scored positive for hepatitis B virus or anti-hepatitis C virus antibodies or had an alcohol consumption exceeding the established limit.

Characteristics of study participants are shown in *Table 1*. 41.5% (412 out of 992 participants) of the subjects were overweight and 27.1% (269) were obese; 47.4% (470) showed central obesity. Overweight was more prevalent in males than in females [48.2% (478) vs 35.4% (351), P < 0.001], but obesity prevalence was similar (P > 0.05). The overall prevalence of NAFLD was 24.8% (246), being higher in males [28.3% (281)] than in females [21.6% (214)]. IFG was reported in

Table 1
Overall prevalence of metabolic and cardiovascular abnormalities in the study participants (992 randomly selected adults aged 18-75 years, Cittanova, Reggio Calabria, Southern Italy, 2009-2011)

Age (years)*	48.0 (17.0-75.0)
BMI (kg/m²)*	27.2 (14.9-52.8)
Overweight (%)	41.5
Obesity (%)	27.1
Waist circumference (cm)*	94.0 (58.0-137.0)
Central obesity (%)	47.4
IFG (%)	34.7
Hypertension (%)	53.7
Low HDL cholesterol (%)	34.2
Hypertriglyceridemia (%)	27.2
NAFLD total (%)	24.8
MS (%)	34.4

* For quantitative variables: median (range). For the other variables: percentage. BMI: body mass index; HDL: high-density lipoprotein; IFG: impaired fasting glucose; MS: metabolic syndrome; NAFLD: non-alcoholic fatty liver disease.

34.7% (344); 53.7% (533) suffered from hypertension; 34.2% (339) had low HDL cholesterol; 27.2% (270) had hypertriglyceridemia; 34.4% (341) had MS. A median of 0.71 mm (range, 0-49-1.59) of IMT was determined in a subset of 344 subjects.

Table 2 shows the PRs of metabolic and cardiovascular risk factors and abnormalities according to presence of NAFLD and without the 125 subjects that scored positive for hepatitis B virus or anti-hepatitis C virus antibodies or that had a daily alcohol consumption exceeding the predetermined limits. In this analysis, we used BMI because of it being the most commonly used measure of body weight and the most useful for comparative purposes. All known cardiovascular risk factors were found in association with NAFLD. When we investigated the PRs of BMI, MS, and IMT adjusted for other variables (BMI, IMT and MS, depending on the PR that was investigated), in addition to age and sex, PRs were still significantly higher for BMI and MS, but not for IMT.

Table 2
Prevalence ratios of the metabolic or cardiovascular risk factors or abnormalities in subjects with NAFLD compared to subjects without NAFLD and without the 125 subjects that scored positive for hepatitis B virus or anti-hepatitis C virus or had a daily alcohol consumption exceeding the predetermined limits (867 subjects)

Cardiometabolic risk factors	PR (95% confidence interval)
BMI (kg/m²)	1.10 (1.09-1.12)* 1.09 (1.06-1.11)**
IMT	2.83 (1.12-7.14)* 1.61 (0.62-4.19)**
Central obesity	2.69 (2.14-3.37)*
IFG and/or antihyperglycemic therapy	1.99 (1.58-2.50)*
Hypertension	2.24 (1.68-3.00)*
Low HDL cholesterol	1.64 (1.34-2.01)*
Hypertriglyceridemia	1.73 (1.39-2.15)*
MS	2.85 (2.26-3.59)* 1.68 (1.23-2.29)**

* Adjusted for age and sex. ** Adjusted for age, sex and other variables (BMI, IMT and MS, depending on the PR that was investigated). BMI: body mass index; HDL: high-density lipoprotein; IFG: impaired fasting glucose; IMT: intima-media thickness; MS: metabolic syndrome; NAFLD: non-alcoholic fatty liver disease; PR: prevalence ratio.

DISCUSSION

This is the first study that has been performed on NAFLD prevalence in an open population of apparently healthy subjects in Southern Italy. The only other Italian study on NAFLD in Italy in a similar population was done in a small city in Northern Italy [11], and was conducted between January 2002 and August 2003. The overall prevalence of NAFLD in that study was 20%, a value lower than the 24.8% found in our study. Both values are consistent with NAFLD prevalence reported in Western countries (20-30%) [3]. As found in the previous Italian study, also in our study NAFLD was associated with MS components, and with MS itself. Regarding overweight and obesity, the values are higher than those in the general Italian adult population [12], and we had already observed a significantly higher prevalence of overweight/obesity in adults from Southern versus Northern Italy.

In our study, after adjustment for several variables in addition to age and sex, the PRs for BMI and MS with regard to the presence of NAFLD remained higher, while the association of NAFLD with increased IMT disappeared. This suggests that the presence of NAFLD is not independently associated with increased carotid IMT, and that other cardiovascular risk factors may be more important in playing a causal role. Conflicting results have been reported about the association of NAFLD with increased IMT.

For example, in a multi-ethnic study, NAFLD was significantly associated with IMT, but the association lost significance after adjustment for BMI and waist circumference [13]. In a study in diabetic patients, no association was found [14]. On the other hand, a recent systematic review and meta-analysis suggested that NAFLD is a strong, independent predictor of cardiovascular disease [15]. Similarly, a Chinese study showed that NAFLD was positively associated with increased carotid IMT, independently of conventional risk factors and of MS [7]. A study in a large European population [16] showed that the association between NAFLD and carotid IMT, was independent of traditional risk factors and MS components. Among the possible reasons for these conflicting results are differences in the prevalence of effect modifiers, and differences in confounders adjusted for. Eventually, one should also consider the possibility that aspects peculiar to this study population, such as dietary elements, might dissociate the relation between NAFLD and IMT once NAFLD is associated with MS and its individual components. Whatever the explanation(s), most of the results that have been reported, including ours, suggest that NAFLD may

be a useful marker for suspecting the presence of early atherosclerosis and, consequently, putting in place diagnostic and prophylactic measures that may be useful to prevent pathologic consequences.

Regarding the role that NAFLD may play, either directly indirectly, alone or in combination with other risk factors, in the pathogenesis of atherosclerosis and related cardiovascular diseases, it is known that obesity and atherosclerosis are precursors of endothelial dysfunction, coronary microvascular dysfunction and coronary artery disease. All of the latter may be promoted by genetic factors [17-20]. It is tempting to speculate that in patients with NAFLD, the presence of these genetic factors may also be involved in determining similar pathologic sequelae. It would be interesting to analyze this possibility in forthcoming studies.

Overall, our results confirm the close association between NAFLD and both BMI and MS, thus, suggesting that once NAFLD is identified, individuals should undergo clinical examinations aimed at identifying silent and/or undiganosed metabolic and cardiovascular diseases. Moreover, it appears highly desirable to complement the present study with a longitudinal investigation in order to disclose the actual relationship between NAFLD and actual pathologic outcomes.

Author's contribution statement

Study aim and design: CA Caserta, A Mele, F Marcucci, M Zuin. Data collection, quality control, data analysis: P Surace, L Ferrigno, A Messineo, C Vacalebre, F Amato, D Baldassarre, M Amato, F Marcucci, M Zuin, CA Caserta, A Mele. Writing the paper: CA Caserta, F Marcucci.

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Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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