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CLINICAL RESEARCH

Fatty liver, carotid disease and gallstones: A study of age-related associations

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Abstract

AIM: To evaluate carotid intima-media thickening (IMT) and plaques, gallstone disease (GD) and fatty liver (FL) as a function of age.

METHODS: In 449 subjects, FL and carotid disease were assessed ultrasonographically. In a subgroup of 65/449 patients with non-alcoholic fatty liver disease (NAFLD), carotid disease, GD and associated factors were determined.

RESULTS: FL of unspecified etiology was more common in younger and GD in older individuals. FL subjects had an increased prevalence of IMT and a decreased prevalence of plaques and manifested carotid disease earlier. Plaques were more common in subjects with GD. Age was an independent predictor of carotid disease outcome and FL was a protective factor for plaques. In NAFLD, there was an inverse correlation between body weight and age and the latter independently predicted carotid findings.

CONCLUSION: Cardiovascular risk in patients with FL and NAFLD needs to be assessed as a function of age and body weight.

Key words: Atherosclerosis; Carotid; Gallstones; Intima-media thickening; Fatty liver

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INTRODUCTION

Atherothrombosis (AT), fatty liver (FL) and gallstone disease (GD) represent a clinically heterogeneous group of disorders which commonly occur in the general population and impose a heavy economic burden for direct and indirect health expenditures[1-4]. Given that insulin resistance is an acknowledged risk factor for their development[5-7], it is expected that AT, non-alcoholic fatty liver disease (NAFLD), one of the most common etiologies of fatty liver, and GD might often affect the same individuals. Several studies have suggested a link between NAFLD and endothelial dysfunction, carotid intima-media thickening (IMT) and carotid plaques[8-10]. However, the development of AT is a complex process involving endothelial dysfunction and IMT as early lesions. IMT, in particular, does not represent definite AT disease but rather it is a marker for its development[10]. In addition, some previous studies may not be representative of NAFLD due to enrollment of only male patients in a narrow range of body mass index (BMI) and age[11,12]; therefore, the occurrence of increased cardiovascular risk in NAFLD remains a controversial issue[13,14].

NAFLD patients might theoretically be prone to GD *via* impaired gallbladder motility and increased bile lithogenicity. Both physiopathologic mechanisms occur in type 2 diabetes mellitus (T2DM) and obesity, which are often associated with NAFLD[7]. However, the NAFLD-GD association remains uncertain given that it has been reported in some studies but rejected in others[15-18]. Furthermore, the association between AT and GD has been reported[19-21], but some authors argue this association might be spurious and influenced by confounders[22]. Although they share a common pathogenetic mechanism, AT, GD and NAFLD occur to a different extent in various age groups. For instance, increasing age is a risk factor for the development of AT and GD[1,4] but a protective factor for NAFLD[23]. Given that no previous studies have evaluated simultaneously the occurrence of AT, GD and FL, we evaluated the patterns of age distribution of FL of

unspecified etiology and its association with carotid disease and GD in either gender across a wide range of age-groups. In addition, we also assessed the independent predictors of carotid disease in a subgroup of patients with definite NAFLD.

MATERIALS AND METHODS

Patients

The electronic database of ultrasound reports for in- and out-patients examined at Modena City Hospital (Operating Unit Internal Medicine and Gastroenterology, to whom one of the authors belonged at the time when data were collected) was searched. Criteria for inclusion in the study were to have undergone both liver and carotid ultrasonographic assessment in the same day or, in any case, not later than 12 mo apart. Focal liver lesions, ascites or other ultrasonographic stigmata of portal hypertension (such as ascites, splenomegaly, presence of patent umbilical vein, varices in the hepato-gastric ligament or spontaneous spleno-renal shunts) were criteria for exclusion. Approximately 1200 liver ultrasound examinations per year were recorded in the 1993-2005 period of time; out of them, all 449 subjects fulfilling the enrollment criteria were selected.

In 384 of 449 cases, information on gender, age, presence/absence of FL and evaluation of carotid morphology was available. No information on being in- or out-patient, final diagnosis, etiology of FL or anthropometric and biochemical data was included in the software of the electronic database. Sixty-five of 449 subjects were part of the series recruited into the POLISTENA study and thus their bright livers were due to NAFLD. In this sub-group, in addition to the previous parameters including liver and carotid US evaluation, a complete history, anthropometric parameters and metabolic data were also available as a part of the protocol of the POLISTENA study[24,25].

In the entire population of 449 subjects, we evaluated the prevalence of FL of unspecified etiology and its relationship with the US carotid findings and GD. In the subgroup of 65 NAFLD patients, the prevalences of carotid alterations, GD and the associated factors were determined.

Parameters evaluated in the whole population

Fatty liver of unspecified etiology: The presence of FL was evaluated through ultrasound scanning with a 3.5 MHz commercially available transducer and defined by the main criterion of "bright" liver as described elsewhere[26]. Additional diagnostic criteria considered were vessel blurring and posterior attenuation of the ultrasound beam. Ultrasound was performed by trained physicians who were unaware of the results of the carotid evaluation. The evaluation of presence/absence of FL among the various operators was evaluated using Chi-square test and no significant difference was found.

Gallstone disease: GD was defined by the presence of one or more echogenic, distal acoustic shadowing, possibly moveable structures in the gallbladder or empty gallbladder fossa in a subjects with a history of cholecystectomy[7].

Carotid intima-media thickening and plaque: Carotid ultrasound evaluation was performed by a single trained operator who was blind to the clinical features of patients. Repeated measurements on the same subject gave coefficients of variation within 5%. The imaging unit was a commercially available machine (AU 600, Hitachi Medical Co., Tokyo, Japan) equipped with a 5-MHz linear array commercially available transducer. The pulsed Doppler frequency was 4.0 MHz. The imaging protocol[27] involved scanning along the entire course of the cervical carotid artery from the supraclavicular notch cephalad to the angle of the mandible. Examination of the right and left common and internal carotid arteries was also performed. The maximum wall thickness observed for either side was taken into consideration. Findings were classified as: (1) normal (intima < 1 mm); (2) intimal thickening (intima-media measuring $\geqslant 1$ mm and < 1.3 mm); and (3) plaque (focal widening of the vessel wall relative to adjacent segments $\geqslant 1.3$ mm) or complicated plaque (any plaque narrowing $> 70\%$ of the lumen).

Parameters evaluated in the subset of patients with NAFLD

Diagnosis of NAFLD: The diagnosis of NAFLD in the subgroup of 65 patients enrolled in the POLISTENA study was based on: (1) absent-to-low alcohol consumption ($\leqslant 30$ g alcohol daily for men and $\leqslant 20$ g for women); (2) evidence of FL at ultrasound scanning as detailed above; and (3) absence of alternative etiologies of chronic liver disease, notably viral, autoimmune, thyroid, drug-induced, hemodynamic and genetic-metabolic (alpha1-antitrypsin, hemochromatosis, Wilson disease). In addition to abdominal ultrasonography and ultrasound carotid scanning, all patients underwent a questionnaire, physical examination and blood sampling for biochemical analysis. In 23 of 65 subjects, liver biopsy was clinically indicated on the grounds of hepatomegaly and/or particularly altered liver function tests and was performed under ultrasound guidance.

Questionnaire: The questionnaire investigated family and personal history, including concurrent diseases, previous surgery, past and current use of medications, possible contacts with toxic agents. Alcohol intake, smoking habits and physical activity were also recorded. Alcohol consumption was assessed through separate interviews with the patient, the referring physician, and family members. Subjects were classified as never smokers (never smoked currently or in the past 5 years) or current smokers. The latter group comprised those smokers of as much as one cigarette a day and also included those ex-smokers who had quitted tobacco since less than 5 years. Physical activity was defined as any exercise, such as jogging, swimming, attending a gymnastics course, cycling, playing tennis, dancing or heavy gardening. Based on their leisure time physical activity, subjects were classified as sedentary (those exercising less than 1 h per week) or non-sedentary (all the others).

General physical examination: It was performed by one of the senior authors. Anthropometric parameters and blood pressure measurements were performed by a trained nurse. Waist girth was measured at the smallest circumference between the ribs and the iliac crest and hip at the maximum circumference between the iliac crest and the crotch. Waist-hip ratio (WHR) was calculated as waist circumference/hip circumference.

Laboratory tests: Laboratory evaluation included renal and liver function tests, blood cell count, total and fractionated proteins, parameters of iron metabolism (serum iron, transferrin and ferritin), lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, apo-A, apo-B), fasting glucose, fasting insulin, and uric acid. Serum concentrations of copper, ceruloplasmin and alpha1-antitrypsin were performed to exclude Wilson disease and alpha1-antitrypsin deficiency. Non-organ-specific autoantibodies were also evaluated. In all patients, the presence of viral serologic markers for B and C infection was assessed by standard methods. Insulin resistance was calculated according to the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index computed through the formula: fasting serum insulin (μU/mL) × fasting serum glucose (mmol/L)/22.5[28]. The diagnosis of impaired glucose tolerance and diabetes was based on the American Diabetes Association criteria[29]. Subjects with impaired glucose tolerance included individuals with fasting glucose levels ⩾ 6.1 mmol/L but < 7 mmol/L and with a 2-h post-load glucose ⩾ 140 mg/dL (7.8 mmol/L) and < 200 mg/dL (11.1 mmol/L). Diabetes prevalence was based on the number of individuals with fasting plasma glucose ⩾ 126 mg/dL (7 mmol/L) checked twice or having a past history of diabetes[29]. Body mass index (BMI) was calculated with the formula: mass (kg)/height $(m)^2$ and obesity was defined by BMI ⩾ 30 kg/m^2. Normal limits for blood lipids were defined accordingly to the third report of the National Cholesterol Education Program (NCEP). Hypercholesterolemia was defined as serum cholesterol ⩾ 5.10 mmol/L, hypertriglyceridemia as serum triglycerides ⩾ 1.65 mmol/L[30]. Hypertension was diagnosed if patients had a past history of hypertension, were taking antihypertensive drugs or had a blood pressure ⩾ 140/90 mmHg.

Liver biopsy: Sections were stained with H&E; silver stain for reticulin, Sirius red for collagen, periodic acid Schiff for glycogen, periodic Schiff-diastase for glycoproteins, and Perl's for iron. Liver biopsy specimens were considered to be adequate if 6 or more portal tracts were included. Evaluation of steatosis and fibrosis was performed following the criteria developed by Brunt *et al*[31]. A diagnosis of NASH required the following features: steatosis, parenchymal inflammation and ballooning degeneration of hepatocytes with or without fibrosis and Mallory bodies[32]. Informed written consent was obtained from all participating individuals before taking blood samples and prior to liver biopsy. The study was performed in agreement with the Declaration of Helsinki.

Statistical analysis

Data were expressed as mean ± SE for variables normally distributed and as median (25^{th}-75^{th} percentile) for those not normally distributed. Normalization of age was achieved through square transformation. The following tests were used as appropriate: Chi-square, Mann-Whitney, Oneway Scheffe's test, ANOVA, linear regression, binary logistic regression. Given that vascular carotid disease may manifest three different features (normal findings, IMT and plaques), we chose to adopt multinominal logistic regression which enables us to keep this distinction in carotid ultrasonographic findings. The software used was SPSS Inc. Chicago, Illinois USA, release 14.0.

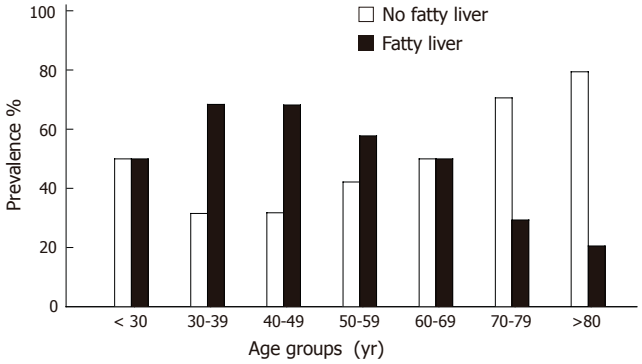

Figure 1 Distribution of ultrasonographically diagnosed fatty liver of unspecified etiology in the various age-groups of adulthood. The number of patients enrolled in each age-group is as follows: < 30: 8 cases; 30-39: 19 cases; 40-49: 44 cases; 50-59: 64 cases; 60-69: 98 cases; 70-79: 119 cases; and >80: 97 cases.

RESULTS

Data analysis in the whole population

Of the 449 subjects fulfilling enrollment criteria, 203 (45.2%) were men and 246 (54.8%) women; the median of the age was 69 years (56.5-78 years). Men were younger [median: 66 years (53-76 years)] than women [median: 72 years (59-81.25)] (Mann-Whitney test, Z = - 3.419, P = 0.001).

Fatty liver of unspecified etiology

FL was present in 42.1% (189/449) of subjects, and the majority (102/189, 54.0%) were males (χ^2 = 10.104, P = 0.0014). The distribution of FL per age-group is shown in Figure 1. The median age of the subjects with FL was 62 years (49-72 years) *versus* 75 years (65-81.75) in those without FL (Mann-Whitney test, Z = -7.479, $P = 7.52 \times 10^{-14}$). Logistic regression analysis using FL as the dependent variable and age and sex as predictors disclosed OR = -0.956, 95% CI = 0.943-0.969 ($P = 2.12 \times 10^{-10}$) for age and OR = -0.631, 95% CI = 0.422-0.942 (P = 0.024) for sex, demonstrating that FL is more common in younger male individuals.

Gallstone disease

The overall prevalence of GD was 32.5% (146/449), not significantly different in patients with FL *versus* those without FL (28.6% *vs* 35.2%; χ^2 = 2.315, P = 0.128). Patients with GD were older than those without GD [73 years (61-82 years) *vs* 68 years (53-77 years); Mann-Whitney test, Z = -3.497, P = 0.00047] and GD was present more often in women than in men (38.6% *vs* 25.1%; χ^2 = 9.230, P = 0.002). Logistic regression analysis using GD as the dependent variable confirmed that advanced age and female gender rather than steatosis predict the presence of GD (OR for age: 1.024, 95% CI: 1.009-1.039, P = 0.002; OR for female gender: 1.698, 95% CI = 1.118-2.578, P = 0.013). Fifty-four subjects had concurrent FL and GD. Compared with subjects with FL alone, these patients had more advanced median age [64 years (53-73 years) *vs* 60

Table 1 Ultrasonographic carotid findings in subjects with or without fatty liver of unspecified etiology

Carotid findings	Fatty liver		
	Absent *n* (%)	Present *n* (%)	*P*
Normal	19 (7.3)	39 (20.6)	
IMT	27 (10.4)	45 (23.8)	
Plaques	214 (82.3)	105 (55.6)	$P = 4.65 \times 10^{-10}$

χ^2 test.

Table 2 Median age and 25th-75th percentile of subjects with/without fatty liver of unspecified etiology (classified according to carotid findings)

Carotid findings	Fatty liver	
	Absent *n* (%)	Present *n* (%)
Normal	44 (31-52)	46 (34-58)
IMT	65 (51-71)	56 (48-62.5)
Plaques	76 (68.75-82)	68 (60.5-78)

Table 3 Carotid ultrasonographic findings in subjects with or without gallstone disease

Carotid findings	Gallstone disease		
	Absent *n* (%)	Present *n* (%)	*P*
Normal	48 (15.8)	10 (6.8)	
IMT	51 (16.8)	21 (14.4)	
Plaques	204 (67.3)	115 (78.8)	$P = 0.015$

χ^2 test.

years(48-71 years); Mann-Whitney test, $Z = -2.121$, $P = 0.034$], but there was no female gender prevalence (females 55.6% *vs* males 44.0%; $\chi^2 = 2.76$, $P = 0.097$).

Carotid ultrasonographic-Doppler evaluation

Fifty-eight of 449 subjects (12.9%) had normal carotid ultrasonographic findings. IMT was found in 16.0% (72/449) of subjects; the prevalence of atherosclerotic plaques was 71% (319/449). The distribution of carotid lesions per age-group is shown in Figure 2. All 8 subjects aged < 30 years had normal carotid findings (Figure 2). Carotid ultrasonographic-Doppler evaluation showed that median age was 45 years (33.75-55.25) for normal, 58 years (48.25-65) for IMT, 75 years (65-81) for plaques (Scheffe's Oneway on squared age $F = 134.118$; $P = 2.48 \times 10^{-46}$).

Relationship between FL of unspecified etiology, GD and carotid ultrasonographic-doppler findings

Table 1 summarises carotid findings as a function of presence/absence of FL. The frequency of subjects with IMT was more than two-fold in those with FL than in those without FL (23.8% *vs* 10.4 %). In contrast, the prevalence of plaques was decreased in those with FL of unspecified etiology than in those without it (55.6% *vs* 82.3%; $\chi^2 = 38.373$, $P = 4.65 \times 10^{-10}$).

Table 2 shows median age and 25th-75th percentile in subjects with/without FL as a function of carotid findings.

Table 4 Independent predictors of intima-media thickening (IMT) or plaques in fatty liver of unspecified etiology

Carotid findings		B	Standard error	*P*	Exp (B) (95% CI)
IMT	Intercept	-3.736	0.916	0.000	
	Male	-0.019	0.405	0.962	0.981 (0.443-2.169)
	Age	0.079	0.016	0.0000014	1.082 (1.048-1.117)
	Fatty liver	0.093	0.426	0.827	0.911 (0.395-2.101)
Plaques	Intercept	-7.879	1.050	0.000	
	Male	0.042	0.403	0.1719	1.719 (0.781-3.786)
	Age	0.166	0.018	2.27×10^{-20}	1.181 (1.040-1.223)
	Fatty liver	0.873	0.416	0.036	0.418 (0.185-0.945)

Multivariate multinominal logistic regression. Dependent variable: carotid findings (IMT or plaques). Reference category: normal carotid findings. Age, sex (forced entry terms), fatty liver and GD (forward entry term) were entered as covariates.

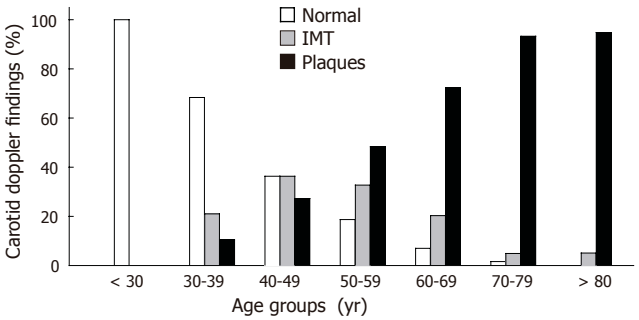

Figure 2 Distribution of carotid ultrasound findings in the various age-groups of adulthood. The number of patients enrolled in each age-group is the same as reported in Figure 1.

Following normalization obtained through square elevation, age was compared through ANOVA. Carotid findings ($F = 114.457$, $P = 8.47 \times 10^{-41}$) and FL ($F = 6.311$, $P = 0.012$) rather than gender (data not shown, $F = 1.529$, $P = 0.217$) were statistically different. Subjects with GD had a higher prevalence of plaques than those without plaques (78.8% *vs* 67.3%; $\chi^2 = 8.351$, $P = 0.015$) (Table 3). In order to determine those variables that were predictive of plaques or IMT, multinominal logistic regression was used. Carotid findings were the dependent variable (normal as the reference value) and age and gender as fixed covariates; factors were FL and GD (forward). As shown in Table 4, age turned out to be an independent predictor of ultrasonographic carotid outcome, OR for IMT being 1.082 (95% CI: 1.048-1.117, $P = 1.40 \times 10^{-6}$) and for plaques 1.181 (95% CI: 1.140-1.228, $P = 2.27 \times 10^{-20}$). The presence of FL was a protective factor for plaques (OR = 0.418, 95% CI: 0.185-0.945, $P = 0.036$) rather than for IMT (OR = 0.911, 95% CI: 0.443-2.169, $P = 0.827$).

Data analysis of the subset of patients with NAFLD

In 65 of 449 subjects belonging to the POLISTENA

Table 5 Historical, anthropometric and laboratory findings of subjects with NAFLD as a function of ultrasonographic carotid findings (mean ± SE, 25th-75th percentile)

Variable	Normal (*n*° 19)	IMT (*n*° 30)	Plaques (*n*° 16)	*P*
Age (yr)	46.11 ± 2.66	55.23 ± 1.59	58.69 ± 2.67	0.001
Gender (M/F)	10/9	13/17	8/8	NS
Smokers	8/17	10/30	6/16	NS
Physical exercise	5/17	11/30	6/16	NS
BMI (kg/m^2)	31.05 ± 1.40	28.85 ± 0.68	27.30 ± 0.85	NS
Mass (kg)	87.21 (76.00-100.00)	79.9 (71.00-88.50)	74.28.50 (66.50-84.00)	0.026
Waist (cm)	102.53 ± 2.73	96.81 ± 2.02	95.31 ± 2.19	NS
Hip (cm)	114.79 ± 3.31	109.30 ± 1.52	107.31 ± 1.44	NS
W/H ratio	0.90 ± 0.02	0.89 ± 0.01	0.89 ± 0.02	NS
Arterial pressure (mmHg)	D: 80.0 (70.0-80.0) S: 120.0 (120.0-130.0)	D: 80.0 (80.0-80.0) S: 130.0 (120.0-130.0)	D: 80.00 (80.00-87.5) S: 130.0 (120.0-145.0)	NS
Fasting glucose (mmol/L)	5.55 (4.75-6.54)	5.39 (5.05-5.83)	5.28 (4.99-5.65)	NS
Uric acid (μmol/L)	345.29 ± 21.76	333.52 ± 14.11	315.88 ± 18.23	NS
Total cholesterol (mmol/L)	5.37 ± 0.26	4.15 ± 0.21	5. 91 ± 0.18	NS
HDL Cholesterol (mmol/L)	1.20 ± 0.06	1.25 ± 0.06	1.21 ± 0.06	NS
Triglycerides (mmol/L)	1.46 (0.99-2.05)	1.49 (1.18-1.98)	1.64 (1.49 - 2.00)	NS
AST (IU/L)	26.00 (23.00-35.00)	25.00 (21.00-31.50)	23.50 (19.50-29.50)	NS
ALT (IU/L)	53.00 (35.00-74.00)	39.00 (29.50-54.00)	36.00 (23.25-40.00)	NS
GGT (IU/L)	40.00 (29.00-71.00)	30.00 (21.00-71.00)	33.00 (24.25-49.25)	NS
Fasting insulin (pmol/L)	119.82 (86.81-145.65)	108.70 (79.28-164.88)	80.71 (60.62-109.77)	NS
Insulin 120 min post-load (pmol/L)	499.38 (269.78-1442.18)	767.72 (324.31-1379.39)	554.26 (383.50-770.45)	NS
APO A (mg/dL)	139.80 ± 7.73	144.58 ± 4.74	137.64 ± 6.03	NS
APO B (mg/dL)	98.00 ± 6.61	111.15 ± 5.04	110.73 ± 6.39	NS
Iron (μmol/L)	16.34 ± 1.04	16.58 ± 0.89	15.99 ± 1.39	NS
Ferritin (mg/dL)	122.68 ± 23.64	129.00 ± 17.95	175.36 ± 30.22	NS
Transferrin saturation (%)	25.99 ± 1.72	29.33 ± 1.80	28.17 ± 2.86	NS
Fibrinogen (g/L)	3.16 ± 0.18	3.53 ± 0.14	3.33 ± 0.22	NS
HOMA-IR index	4.207 (2.936-5.941)	3.864 (2.708-5.869)	2.815 (2.256-3.861)	NS
Lp(a) (mg/dL)	35.00 (8.50-77.00)	12.00 (4.00-30.00)	9.00 (5.50-28.5)	NS

Significance of the comparisons evaluated through oneway Scheffe's test for variable normally distributed, and Kruskall Wallis for those not normally distributed.

Table 6 NAFLD. Hepatic histological findings in NAFLD patients with normal carotid findings, intima-media thickening (IMT) and atherosclerotic carotid plaques[1]

Hepatic histology	Carotid findings			
	Normal *n* (%)	IMT *n* (%)	Plaques *n* (%)	*p*
Inflammatory grade				
0	2 (29)	1 (10)	2 (33)	
1	3 (43)	5 (50)	2 (33)	
2	2 (29)	2 (20)	2 (33)	
3	0 (0)	2 (20)	0 (0)	0.516[2]
Fibrosis stage				
0	5 (71)	7 (70)	5 (83)	
1	1 (14)	1 (10)	1 (17)	
2	1 (14)	2 (20)	0	
3	0	0	0	
4	0	0	0	0.762[2]
Steatosis extent (%)	45.8 ± 9.9	51.0 ± 8.9	23.00 ± 5.4	0.131[3]

[1]23 of 65 NAFLD patients underwent liver biopsy; 7 had normal findings, 10 IMT, and 6 carotid plaques. [2]Kruskall-Wallis test; [3]One way analysis of variance.

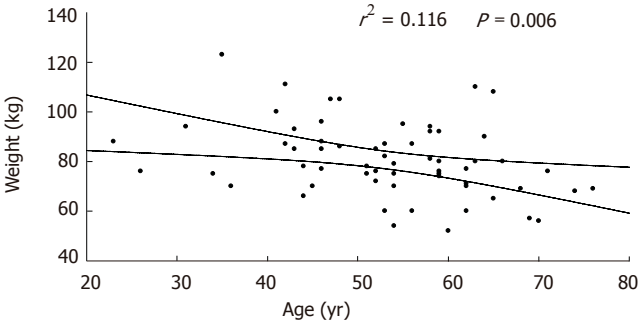

Figure 3 Inverse correlation between body weight and age in NAFLD patients.

study[23,24], ultrasound carotid examination was also performed. These patients had an average age of 53.42 ± 1.38 years. Thirty-one of 65 (47.7%) were males with an average age of 48.06 ± 2.03 years, which was significantly lower than the age observed in women 58.29 ± 1.44 years (t = -4.159, P = 0.00001). The prevalence of components of the metabolic syndrome was as follows: hypercholesterolemia 72.3% (47/65); arterial hypertension 38.4% (25/65); obesity 36.9% (24/65); hypertriglyceridemia 33.85% (22/65); IGT 27.7% (18/65); and T2DM 21.5% (14/65). Out of 65 patients, 10 (15.4%) had GD. These GD patients were older than those without GD (59.00 ± 1.65 years *vs* 52.40 ± 1.56; t = -2.91, P = 0.007), with a similar proportion of males and females (9.68% *vs* 20.59%; Fisher's exact test, P = 0.309).

Patients with normal carotid findings were younger than those with IMT and with plaques (Oneway Scheffe's test, F = 7.652, P = 0.001). Individuals with plaques had lower body weight (Oneway Scheffe's test, F = 3.878, P = 0.026) and transferrin serum levels (Oneway Scheffe's test, F = 4.491, P = 0.015) than those with normal carotid findings (Table 5), indicating a significant inverse relationship (r^2 = 0.116; P = 0.006) between age and body weight (Figure 3). We also found a direct relationship between HOMA and BMI (r^2 = 0.26, P = 0.000017) rather than between HOMA and age. No relationship was found between histological variables and carotid ultrasonographic findings in the limited number of cases available (Table 6).

The distribution of carotid ultrasonographic findings in the various age-groups of adulthood is shown in Figure

Table 7 Independent predictors of intima-media thickening (IMT) or plaques in NAFLD subjects

Carotid findings		B	Standard error	P^1	Exp (B) (95% CI)
IMT	Intercept	-0.955	2.648	0.718	
	Age	0.087	0.034	0.010	1.091 (1.021-1.165)
	BMI	-0.102	0.071	0.150	0.903 (0.785-1.038)
Plaques	Intercept	-0.913	3.628	0.801	
	Age	0.119	0.042	0.005	1.126 (1.037-1.223)
	BMI	-0.189	0.097	0.052	0.828 (0.684-1.002)

[1]Multivariate multinominal logistic regression. Dependent variable: carotid findings (IMT or plaques). Reference category: normal carotid findings. Age, body mass (forced entry terms) and HOMA (forward entry term) were entered as covariates.

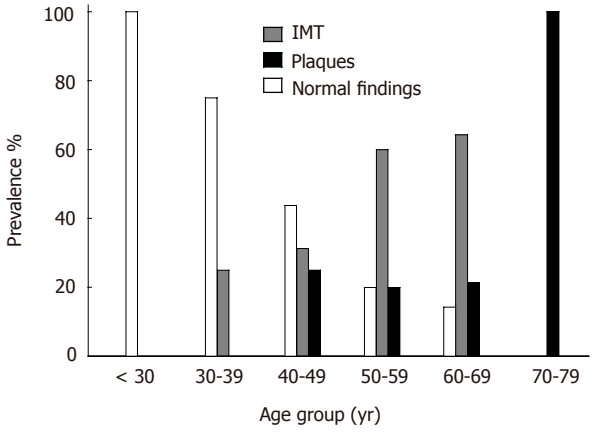

Figure 4 Distribution of carotid ultrasound findings in the various age-groups of adulthood with NAFLD. The number of patients enrolled in each age-group is as follows: < 30: 2 cases; 30-39: 4 cases; 40-49: 16 cases; 50-59: 25 cases; 60-69: 14 cases; and 70-79: 4 cases.

4. Variables associated with IMT and plaques (the reference being normal findings) were evaluated using univariate logistic multinominal regression, where age, BMI and HOMA were found to be significant independent predictors (data not shown). In the multivariate logistic multinominal regression (Table 7), only age turned out to be the significant independent predictor of carotid IMT (OR: 1.091, 95% CI: 1.021-1.165, $P = 0.010$) and plaques (OR: 1.126, 95% CI: 1.037-1.223, $P = 0.005$).

DISCUSSION

To the best of our knowledge, this is the first study which explores the prevalence and determinants of carotid disease, FL and gallstones. A major finding of this study was that distribution of FL per age-classes (Figure 1) peaked in the 30-49 years age-group and declined in the younger and more advanced age groups. This "inverted U" pattern resembles that reported for FL distribution in the general population in Japan[15] and closely mirrors the curve of altered transaminase levels in USA[33,34] and in Israel[35]. Taken collectively, these data further support the theory that FL, most likely NAFLD, accounts for the vast majority of altered liver function tests (LFTs) in the Western world. We have no explanation as to why the prevalence of FL of unspecified etiology declined after the age of 49 years. Several hypotheses can be put forward. The phenomenon may reflect a simple decrease in risk factor for NAFLD, notably including obesity, as supported by the finding in the present study that body weight declines with age (Figure 3). An alternative explanation is that mortality might be selectively increased among those with FL of unspecified etiology. A recent study has shown that the presence of NAFLD is associated with an increased mortality as compared with the general population[36]. A unifying explanation could be that only those NAFLD subjects who lose weight will survive till more advanced age. This hypothesis, though, remains speculative and needs support from prospective studies.

Relevant findings of this study are that subjects with FL of unspecified etiology had an increased prevalence of IMT and a decreased prevalence of plaques; in contrast, GD was associated with increased prevalence of plaques. Of interest, carotid disease was found to occur in FL patients approximately 8 to 9 years earlier than those observed in subjects without FL. In FL of unspecified etiology, age was found to be an independent predictor of IMT and plaques, and steatosis had a protective effect in plaques only. Age was also recognized as the only independent predictor of carotid findings in NAFLD.

Although the prevalence of IMT among subjects with FL of unspecified etiology was increased, FL of unspecified etiology was not shown an independent predictor of IMT and was a negative predictor of plaques at logistic regression multinominal analysis; in contrast, GD and carotid plaques were shown to be associated. These findings can all be accounted for by the influence of age on the diseases in study. IMT and FL of unspecified etiology had the same distribution per age-classes, namely decreased in individuals aged > 40 years, whereas carotid atherosclerosis and GD increased in such over-40-year-old subjects (Figure 1). An increased prevalence of IMT among subjects with FL has also been reported in previous studies[9,37]. However, while these authors reported the absolute value of IMT expressed in mm, we used a > 1-1.3 mm cut-off value, namely the threshold level predicting the occurrence of cardiovascular events[38]. These methodological differences imply that we might have underestimated the true prevalence of IMT among subjects with FL of unspecified etiology to the advantage of predicting clinically relevant events. A recent study[39] showed that after adjustment for confounders, NAFLD was significantly associated with an increased risk of cardiovascular diseases. However, additional adjustment for the metabolic syndrome appreciably attenuated this association without abolishing it. The finding that FL of unspecified etiology is an independent negative predictor of carotid plaques is probably linked to the inverse relationship linking age and body weight (Figure 3). Therefore, we emphasize that these data do not support a true "protective" effect of FL of unspecified etiology on the development of carotid plaques but probably represent the effect of selective mortality in those with FL and advanced AT, as suggested by the finding in this study that those with FL and IMT or plaques were 8 to 9 years younger than those with the same carotid findings without

FL.

Our data confirm the association between GD and carotid plaques[20,21] but, in agreement with a previous study[17], challenge that between GD and NAFLD. Both these findings are accounted for by the fact that carotid disease and GD both increase, whereas FL of unspecified etiology decreases, with age.

The importance of distribution per age-groups of subjects with various carotid findings, FL, NAFLD and GD also links the first (FL of unspecified etiology) and the second part (NAFLD) of our study. Individuals with NAFLD were significantly younger than those included in the first part of the study and thus they were less prone to the development of carotid plaques and GD. We highlight that in this subset of patients where all data were available, no other anthropometric, biological or histological parameters except for age, is an independent predictor of IMT and carotid plaques as found in the whole series. This finding fits with a previous study indicating that age is a powerful determinant of maximum IMT in both diabetic and healthy subjects[40].

Some studies suggest that NAFLD patients have an increased incidence of carotid atherosclerosis[9,41,42]. However, there is not enough hard data coming from natural history to support or rule out an association between NAFLD and cardiovascular disease. Indeed, some follow-up studies indicate that excess mortality in NAFLD is due to liver-related causes rather than to cardiovascular events[13,14,36,43]. Enrollment criteria and referral pattern of patients might affect the vascular risk in NAFLD patients. NAFLD individuals included in our series were young and had a low prevalence of arterial hypertension and reduced HDL cholesterol that are strong determinants of AT[44-46]. Furthermore, factors that are associated with the onset of carotid plaques might be different from those that cause clinical events of (complicated) AT.

Some specific features of our study need to be emphasized. First, the large series of subjects who were recruited. These individuals were distributed across all age-groups of adulthood, so providing an exhaustive cross-sectional picture of the relationship between the variables. Furthermore, our study enabled to evaluate those risk factors that add to the background dysmetabolic milieu linked to NAFLD *per se*. Indeed, our "control" group consisted of NAFLD patients with normal carotid findings rather than of subjects with a normal liver (i.e. without FL). Finally, we used the statistical procedure of multinominal logistic regression analysis which is particularly suitable in the analysis of unrelated multiple events, such as the varying outcomes of carotid ultrasonographic findings. We acknowledge, however, that ours is not a prospective study and that absence of anthropometric and biochemical data in the population with FL of unspecified etiology together with the limited number of NAFLD subjects may preclude exhaustive analysis of the etiological link between evaluated variables. Furthermore, the indications for prescribing ultrasound scanning of the liver or carotid depend on the clinical picture displayed by patients rather than by a standardized protocol. This may have reduced the number of subjects included in some age-groups (e.g. those aged 30 to 39 years) because these younger individuals seldom present indications to undergo ultrasound scanning of carotid. This might somewhat bias our findings, that need to be duplicated by future studies.

In conclusion, our data show that in a large population comprising all ages of adulthood, age is an independent predictor of carotid IMT and plaques. FL of unspecified etiology is a negative predictor of carotid plaques, probably indicating selective death of those with FL or disappearance of liver fat with ageing. While we confirm an increased prevalence of IMT among patients with FL of unspecified etiology, and an association of GD with carotid atherosclerosis, we were unable to find any independent relationship between any conventional parameters, such as anthropometric, metabolic, hemodynamic, and histological liver features and carotid disease in NAFLD. In this specific subset of individuals, again, age is the only independent predictor of ultrasonographic carotid findings. Studies of natural history are needed to evaluate the real entity of cardiovascular risk in patients with FL of unspecified etiology and NAFLD as a function of age.

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