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Relationship of Serum Fetuin-A Levels with Coronary Atherosclerotic Burden and NAFLD in Patients Undergoing Elective Coronary Angiography

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Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) patients are prone to coronary artery disease (CAD). Fetuin-A inhibits arterial calcification, induces insulin resistance, and is increased in NAFLD. Data on fetuin-A levels in CAD are conflicting. We tried to ascertain whether NAFLD and CAD are associated and if fetuin-A predicts CAD and/or NAFLD.

Methods: CAD was diagnosed by ≥50% stenosis in coronary arteries and NAFLD by ultrasound imaging in the absence of any other liver disease. Seventy patients who underwent elective coronarography at our hospital were recruited in this cross-sectional study. Twenty-four patients had no CAD (9 with and 15 without NAFLD) and 46 had CAD (20 with and 26 without NAFLD). Standard anthropometric indices and metabolic parameters were recorded. Fetuin-A was determined by enzyme-linked immunosorbent assay (ELISA). Visceral fat thickness and visceral/subcutaneous fat ratio were assessed by ultrasonography.

Results: NAFLD was not associated with CAD, probably owing to the limited series. Fetuin-A was significantly lower, whereas visceral fat thickness and visceral/subcutaneous fat ratio were higher in patients with CAD versus those without CAD. Younger age and higher body mass index (BMI), waist circumference, triglycerides, fasting glucose, homeostasis model assessment, spleen area, subcutaneous fat thickness, and prevalence of metabolic derangements were associated with NAFLD. At multivariate analysis, elevated fetuin-A levels were an independent negative predictor of CAD [odds ratio (OR) = 0.995, $P = 0.049$]. Fetuin-A was an independent predictor of NAFLD (OR = 1.005, $P = 0.036$) in the model including BMI.

Conclusions: This prospective cross-sectional study demonstrates high fetuin-A levels to be independently associated with NAFLD and a lower risk of coronarographically diagnosed CAD.

Introduction

Nonalcoholic fatty liver disease (NAFLD) spanning simple steatosis through nonalcoholic steatohepatitis (NASH) with or without fibrosis/cirrhosis and primary liver cancer[1] is strongly associated with the metabolic syndrome[2] and insulin resistance (IR).[3] Several lines of evidence support NAFLD to be associated with cardiovascular disease (CVD)[4] and increased cardiovascular mortality, mainly due to coronary artery disease (CAD) in humans.[5] The biological mechanisms linking NAFLD with CVD are incompletely understood. However, atherogenic dyslipidemia, systemic and hepatic inflammation, and prothrombotic state are all deemed to be capable of promoting atherogenesis in NAFLD patients.[6] Moreover, IR must play a major role, given that it is an early and almost universal finding in NAFLD and an accepted risk factor for CVD.[7]

Fetuin-A (a_2-Heremans and Schmidt glycoprotein) is an anti-inflammatory negative acute-phase reactant synthesized in the liver that induces IR and inhibits ectopic calcification.[8] Fetuin-A represents an attractive biological candidate to link NAFLD and CVD on the grounds that it is an independent risk factor for type 2 diabetes[9]; it is increased in subjects with fatty liver and metabolic syndrome and is associated with IR/diabetes, atherogenic dyslipidemia, increased inflammatory cytokines, and reduced adiponectin levels.[8] Moreover,

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fetuin-A levels are correlated with liver fibrosis in NAFLD.[10] In summary, fetuin-A seems to play a role in CVD via two effects—promoting metabolic disorders and inhibiting vascular calcifications.[8] Studies in CAD patients are conflicting, some authors indicating low[11–13] and others reporting increased levels[14,15] of fetuin-A in these patients. A recent study reports an independent positive association of fetuin-A levels with endothelial dysfunction and increased carotid intima-media thickness.[16] On the grounds of such data, we hypothesized that fetuin-A might modulate cardiovascular risk in NAFLD and designed this study to ascertain whether NAFLD and CAD are associated and whether fetuin-A is associated with the presence of CAD and NAFLD.

Patients and Methods

Patients

The study population included all ($n=73$) patients consecutively submitted to elective coronary angiography for suspected CAD between April, 2010, and July, 2010, at the Operating Unit (OU) of Cardiology of our hospital.

Patients were referred to our OU of Internal Medicine and Metabolic Disorders to perform an abdominal ultrasound scanning to detect fatty liver within 1 week of coronary angiography. Clinical/anthropometric data were recorded and blood samples drawn. Criteria for the diagnosis of NAFLD at enrollment were presence of "bright liver" at ultrasonography, absent-to-low alcohol consumption (≤ 20 g daily for women and ≤ 30 g daily for men), and absence of other known etiologies of chronic liver disease, notably viral, autoimmune, drug-induced, vascular, and inherited (α1-antitrypsin deficiency, hemochromatosis, and Wilson disease). Three patients with a significant alcohol intake were excluded. On the basis of such criteria, 70 consecutive patients were enrolled in this cross-sectional study.

All patients underwent an interview aimed at investigating their family and personal medical history, including previous surgery, dietary habits, smoking, alcohol consumption, past and current use of medications, possible contacts with toxic agents, or exposure to drugs associated with NAFLD or affecting lipid metabolism. Metabolic co-morbidity was explored by appropriate questions concerning the previous diagnosis of type 2 diabetes and metabolic syndrome and its individual components. A physical examination and blood sampling were performed, and anthropometric parameters and blood pressure measurements were registered.

All patients consented to take part in the study. Informed written consent was obtained from all participating individuals before coronary angiography. The study was performed in agreement with the Declaration of Helsinki. The protocol of this study was approved by the local Ethics Committee.

Coronary angiography (coronarography)

All patients enrolled in the study had selective left and right coronary angiography performed in election in the hemodynamic laboratory of OU of Cardiology using a Brilliance Intensifier General Electric Innova (GE Healthcare, USA).

On the basis of data in the literature,[17–19] the presence of clinically relevant CAD was evaluated according to a 50% or greater diameter stenosis in epicardial coronary arteries or their major branches. Individuals with no stenosis or stenosis $<50\%$ were enrolled as controls.

The Gensini scoring system was used to determine the severity of coronary atherosclerosis.[20] The Gensini score was computed by quantifying coronary stenosis according to the degree of luminal narrowing and its anatomic and topographic importance. Reduction in the lumen diameter and the roentgenographic appearance of concentric lesions and eccentric plaques were evaluated (reductions of 25%, 50%, 75%, 90%, 99%, and complete occlusion were given Gensini scores of 1, 2, 4, 8, 16, and 32, respectively). Each principal vascular segment was assigned a multiplier in accordance with the functional significance of the myocardial area supplied by that segment—the left main coronary artery, $\times 5$; the proximal segment of left anterior descending coronary artery (LAD), $\times 2.5$; the proximal segment of the circumflex artery, $\times 2.5$; the mid segment of the LAD, $\times 1.5$; the right coronary artery, the distal segment of the LAD, the posterolateral artery, and the obtuse marginal artery, $\times 1$; and others, $\times 0.5$.

Ultrasonographic criteria for the diagnosis of NAFLD

Abdominal ultrasound scanning was performed after overnight fasting, by a single expert physician (S.B.), blinded to the biochemical, clinical, and coronarography data of patients, with a 3.5- to 5-MHz convex probe and a high-resolution B-mode scanner (Siemens Sonoline ANTARES^{TM}) in our Unit.

Fatty liver was diagnosed based on increased liver echogenicity compared to renal cortex.[21] The severity of steatosis was evaluated according to the Ultrasonographic-Fatty Liver Indicator (US-FLI), a semiquantitative ultrasonographic score recently proposed by our group.[21] US-FLI ranges from 2 to 8. *"Conditio sine qua non"* for the diagnosis of steatosis is the presence of liver/kidney echo contrast, graduated as mild/moderate (score 2) and severe (score 3). Additional criteria include the presence (score 1 each) or absence (score 0 each) of posterior attenuation of ultrasound beam, vessels blurring, difficult visualization of gallbladder wall or diaphragm, and areas of focal sparing.

Subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) thickness were determined as described by Pontiroli et al.[22] A 3.5- to 5.0-MHz curved array transducer was placed along the xypho-umbilical line next to the umbilicus, and VAT and SAT were measured after gentle breathing out. VAT was measured from the internal surface of the rectus abdominalis muscle to the proximal aortic wall. SAT was measured, at the same anatomic and topographic position, as the distance between the external surface of the muscle and the skin. The thickness of the muscle and skin were not computed.

Laboratory tests

Blood was sampled after an overnight fasting. Laboratory evaluation included blood cell count, alanine and aspartate aminotransferases (ALT, AST), γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), total and direct bilirubin, total and fractionated proteins, international normalized ratio (INR), serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TGs), fasting glucose, insulin, uric acid, creatinine, thyroid-stimulating hormone (TSH), free thyroxine (FT4) and triiodothyronine (FT3). Moreover, in all subjects, other etiologies of chronic liver disease were ruled out by appropriate testing, including

serum concentrations of copper, ceruloplasmin, and α1-antitrypsin, smooth muscle antibody, antinuclear antibody, liver kidney microsome, antimitochondrial antibody, and hepatitis B virus (HBV) and hepatitis C virus (HCV) serology.

Glomerular filtration rate (GFR) (mL/min per 1.73 m^2) was estimated from serum creatinine by the Modification of Diet in Renal Disease (MDRD) Study equation.[23] Fetuin-A concentration was determined by a commercially available kit [Fetuin-A (Alpha2-HS Glycoprotein, AHSG) enzyme-linked immunosorbent assay (ELISA) CE/IVD, 96 wells, BioVendor, Czech Republic]. Intra-assay (within-run) ($n=6$) coefficient of variation (CV %) was 3.9 in sample 1 and 6.5 in sample 2. Interassay (run-to-run) ($n=6$) CV % was 5.1 in sample 1 and 2.6 in sample 2. All serum samples were processed in duplicates after a 1:10,000 dilution.

Metabolic parameters

IR, calculated according to the homeostasis model assessment (HOMA)-IR index,[24] body mass index (BMI) [(kg)/height $(m)^2$], impaired glucose tolerance and diabetes, arterial hypertension, and metabolic syndrome were defined based on standard criteria.[24–27]

Statistical analysis

The Kolmogorov–Smirnov test was used to assess the normality of variables. Results were expressed as mean ± standard deviation (SD) for continuous variables normally distributed, as median (25^{th}–75^{th} percentile) for those not normally distributed (diastolic blood pressure, ALT, AST, GGT, glucose, insulin, HOMA, creatinine, and TSH) and as frequencies (percentages) for categorical variables. Comparisons between the means of continuous variables were performed using the unpaired two-tailed Student *t*-test for normally distributed variables, whereas the Mann–Whitney test was used for non-normally distributed variables. The Fisher exact test was used to compare nominal variables.

Stepwise multivariable logistic regression analyses were performed to identify single independent predictors of NAFLD and CAD (dependent variables). Exp (B) and 95% confidence intervals were reported with *P* values. To choose those variables to be processed at the multivariate analysis, three steps were followed. First, based on generally available literature and on clinical judgment, a certain number of variables were selected. Next, a univariate model was generated. Finally, those variables that were identified as significant predictors of NAFLD or CAD at univariate analysis ($P<0.05$) were entered in the multivariate analysis.

The multivariate analysis to assess the independent predictors NAFLD was performed twice to analyze the importance of two major correlates of NAFLD, BMI, and metabolic syndrome separately. Model 1 included age, gender, and BMI as covariates and processed those variables found to be significant at univariate analysis. Model 2 included age, gender, and metabolic syndrome as covariates and processed those variables found to be significant at univariate analysis.

A two-sided *P* value < 0.05 was considered to be significant. Statistical analyses were performed using the statistical software package SPSS, version 18.0 for Windows (SPSS Inc., IL).

Results

The study population consisted of 70 patients submitted to coronarography: 24 without CAD (9 with and 15 without NAFLD) and 46 with CAD (20 with and 26 without NAFLD). We studied the same clinical, biochemical, and ultrasonographic parameters by two groups of patients, separately: (1) patients with and without CAD were compared first (Table 1) and (2) those with and without NAFLD (Table 2).

Clinical, biochemical, and ultrasonographic features of CAD-positive versus CAD-negative patients

Patients were comparable for age and gender, and the two groups differed statistically for fetuin-A serum levels, VAT thickness, and VAT/SAT ratio (Table 1). Fetuin-A was lower and VAT and VAT/SAT were higher in patients with CAD. The prevalence of NAFLD did not differ statistically by the presence (43.5%) or absence (37.5%) of CAD ($P=0.799$), most likely as a result of the limited number of patients recruited.

The Gensini score did not statistically differ in CAD-positive patients with [16.0 (5.7–49.0)] or without NAFLD [35.0 (12.0–56.0)] ($P=0.287$)].

Clinical, biochemical, and ultrasonographic features of NAFLD-positive versus NAFLD-negative patients

Patients with NAFLD were younger than those without NAFLD. BMI, waist circumference, TGs levels, fasting glucose, HOMA, spleen area, and SAT thickness, as well as the prevalence of metabolic derangements (hyperlipidemia, diabetes, and metabolic syndrome), were significantly higher in NAFLD patients (Table 2). Fetuin-A was higher in patients with NAFLD as compared to those without NAFLD, but the difference was not statistically significant ($P=0.115$).

The clinical significance of a higher spleen area in those patients with NAFLD was explained by linear regression analysis, which disclosed spleen area to be significantly associated with BMI ($\beta=0.661$; $P=0.000$), age ($\beta=-0.586$; $P=0.002$), and waist circumference ($\beta=0.187$; $P=0.027$).

Independent predictors of CAD and NAFLD

To assess the independent predictors of CAD (Table 3) and NAFLD (Table 4), we entered those variables that were statistically significant at univariate logistic regression analysis and those selected based on clinical judgment and literature data into multivariate stepwise logistic regression analysis.

Data have shown that low fetuin-A levels independently predicted CAD in our population (OR = 0.995, $P=0.049$) (Table 3). Fetuin-A (OR = 1.005, $P=0.036$) and BMI (OR = 1.298, $P=0.005$) were independent positive predictors of NAFLD in Model 1 (in which BMI was used as fixed covariate) (Table 4A). However, fetuin-A was no longer significant in Model 2 in which metabolic syndrome, used as fixed covariate, turned out to be the only independent predictor of NAFLD (OR = 3.556, $P=0.022$) (Table 4B).

After excluding diabetic patients ($n=16$), fetuin-A was no longer an independent predictor of CAD and NAFLD at multivariate analysis. The VAT/SAT ratio independently predicted CAD, whereas BMI (in Model 1) and metabolic syndrome (in Model 2) independently predicted NAFLD.

Table 1. Clinical, Biochemical, and Ultrasonographic Characteristics of Patients According to the Presence or Absence of CAD

	CAD-positive (46)	*CAD-negative (24)*	P
Age (years)	67.6 ± 11.4	68.9 ± 13.6	0.686
Male gender, *n* (%)	35 (71.6)	13 (54.2)	0.102
BMI (kg/m^2)	27.4 ± 4.1	26.9 ± 4.6	0.620
Waist circumference (cm)	100.4 ± 11.7	99.4 ± 14.4	0.765
SBP (mmHg)	134.5 ± 15.5	135.4 ± 13.8	0.800
DBP (mmHg)	80.0 (75.0–85.0)	80.0 (76.2–88.7)	0.380
Smoke, *n* (%)	6 (26.1)	17 (37.0)	0.426
Hypertension, *n* (%)	36 (78.3)	15 (62.5)	0.171
Hyperlipidemia, *n* (%)	34 (73.9)	16 (66.7)	0.583
Diabetes, *n* (%)	11 (23.9)	5 (20.8)	1.000
Metabolic syndrome, *n* (%)	22 (47.8)	12 (50.0)	1.000
NAFLD, *n* (%)	20 (43.5)	9 (37.5)	0.799
WBCs count ($\times 10^3/\mu$L)	7.57 ± 2.4	6.75 ± 2.0	0.161
Platelets ($\times 10^3/\mu$L)	193.0 ± 51.0	181.7 ± 48.4	0.377
AST (U/L)	22.0 (18.4–26.0)	22.6 (20.2–31.6)	0.304
ALT (U/L)	26.0 (19.0–36.8)	29.2 (18.2–42.7)	0.435
GGT (U/L)	24.0 (18.0–29.5)	20.2 (15.5–28.8)	0.335
TC (mg/dL)	173.8 ± 31.9	184.8 ± 46.6	0.308
LDL-C (mg/dL)	107.6 ± 31.5	111.7 ± 39.7	0.158
HDL-C (mg/dL)	40.2 ± 11.0	45.2 ± 17.6	0.221
TGs (mg/dL)	130.5 ± 59.8	106.7 ± 52.8	0.106
Glucose 0′ (mg/mL)	98.0 (92.5–114.0)	96.0 (83.2–117.8)	0.371
Insulin 0′ (μIU/mL)	5.3 (3.2–8.1)	4.8 (2.825–6.9)	0.409
HOMA-IR	1.3 (0.8–2.0)	1.1 (0.70–1.7)	0.351
Uric acid (mg/dL)	6.2 ± 1.1	5.9 ± 1.8	0.416
GFR (mL/min/1.73 m^2)	74.8 ± 22.6	68.7 ± 22.8	0.287
TSH (IU/mL)	1.4 (0.9–2.1)	1.3 (0.8–2.1)	0.738
Fetuin (μg/mL)	374.0 ± 123.9	445.8 ± 146.5	0.038
Spleen area (cm^2)	39.6 ± 8.0	44.0 ± 7.4	0.197
SAT (mm)	14.4 ± 5.3	15.2 ± 8.0	0.626
VAT (mm)	61.7 ± 23.9	46.0 ± 27.0	0.020
VAT/SAT ratio	4.7 ± 2.0	3.4 ± 2.0	0.020
Drugs			
Antihypertensive, *n* (%)	36 (78.3)	13 (54.2)	0.054
Lipid-lowering, *n* (%)	31 (67.4)	10 (41.7)	0.045
Hypoglycemic, *n* (%)	10 (21.7)	5 (20.8)	1.000

Data are expressed as mean ± standard deviation (SD) and median (25^{th}–75^{th} percentile) for continuous variables normally and not normally distributed, as frequencies (percentages) for categorical variables. The Student *t*-test was used for normally distributed variables. The Mann–Whitney test was used for non-normally distributed variables. The Fischer exact test was used for proportions.

CAD, coronary artery disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; NAFLD, nonalcoholic fatty liver disease; WBCs, white blood cells; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TGs, triglycerides; HOMA, homeostasis model assessment of insulin resistance; GFR, glomerular filtration rate; TSH, thyroid-stimulating hormone; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

Correlation analysis of fetuin-A, Gensini score, and US-FLI

In the entire study population, we did not find a significant correlation between fetuin-A and metabolic parameters. A negative correlation (Pearson $r = -0.341$) was found between fetuin-A and white blood cells (WBCs) ($P = 0.005$). Consistent with the results of multivariate analysis, fetuin-A was negatively correlated with the Gensini score (Spearman $\rho = -0.292$) in patients with CAD; however, this was borderline significant ($P = 0.064$).

Correlation analysis by the Spearman ρ were performed between US-FLI and clinical and laboratory parameters in patients with NAFLD, and between the Gensini score and clinical and laboratory parameters in those with CAD. US-FLI was correlated with anthropometric indices and metabolic parameters (BMI, $\rho = 0.407$, $P = 0.035$; waist circumference, $\rho = 0.416$, $P = 0.031$; insulin, $\rho = 0.494$, $P = 0.010$) but not with fetuin-A or the Gensini score. The Gensini score was positively correlated with serum glucose levels ($\rho = 0.362$; $P = 0.020$) and negatively with SAT thickness ($\rho = -0.339$; $P = 0.040$).

On the basis of literature data, we compared fetuin-A levels according to diagnosis of diabetes or metabolic syndrome in the entire study population. Fetuin-A levels were higher in diabetics (408.4 ± 40.4) than in nondiabetics (395.88 ± 18.06) as well as in patients with (406.9 ± 22.1) than in those without metabolic syndrome (390.7 ± 24.8). However, in both cases, the differences were not statistically significant ($P = 0.518$ and $P = 0.292$, respectively).

Discussion

Accumulating evidence supports the link between NAFLD and CAD.[28] Mirbagheri et al.[29] in a population of 317 adult patients undergoing elective coronarography were first in

Table 2. Clinical, Biochemical, and Ultrasonographic Characteristics of NAFLD and Non-NAFLD Patients

	NAFLD (29)	*Non–NAFLD (41)*	P
Age (years)	64.5 ± 10.5	70.6 ± 12.7	0.039
Male gender, *n* (%)	20 (69.0)	28 (68.3)	1.000
BMI (kg/m^2)	29.2 ± 5.0	25.8 ± 3.1	0.003
Waist circumference (cm)	104.8 ± 13.5	96.6 ± 10.9	0.007
SBP (mmHg)	135.7 ± 14.7	134.1 ± 15.1	0.672
DBP (mmHg)	80.0 (80.0–90.0)	80.0 (72.5–85.0)	0.121
Smoke, *n* (%)	12 (41.4)	11 (27.5)	0.302
Hypertension, *n* (%)	22 (75.9%)	29 (70.7%)	0.786
Hyperlipidemia, *n* (%)	25 (86.2%)	25 (61.0%)	0.031
Diabetes, *n* (%)	11 (37.9%)	5 (12.2%)	0.019
Metabolic syndrome, *n* (%)	19 (65.5%)	15 (36.6%)	0.028
CAD, *n* (%)	20 (69%)	26 (63.4%)	0.799
WBCs count ($\times 10^3/\mu L$)	7.1 ± 1.7	7.4 ± 2.7	0.625
Platelets ($\times 10^3/\mu L$)	199.1 ± 54.5	182.1 ± 46.1	0.163
AST (U/L)	22.0 (19.0–25.7)	22.0 (19.0–28.6)	0.718
ALT (U/L)	27.5 (19.4–41.8)	26.9 (17.3–39.9)	0.638
GGT (U/L)	22.45 (19.0–27.7)	21.8 (14.6–31.1)	0.501
TC (mg/dL)	186.1 ± 43.1	171.8 ± 32.8	0.123
LDL-C (mg/dL)	116.1 ± 40.9	104.2 ± 28.5	0.188
HDL-C (mg/dL)	40.6 ± 11.8	42.9 ± 15.0	0.493
TGs (mg/dL)	140.9 ± 63.9	109.4 ± 50.9	0.026
Glucose 0′ (mg/mL)	101.0 (89.5–127.5)	96.0 (88.5–105.5)	0.171
Insulin 0′ (μIU/ml)	5.7 (3.7–8.7)	4.3 (2.7–6.5)	0.134
HOMA-IR	1.5 (1.0–2.3)	0.9 (0.6–1.6)	0.042
Uric acid (mg/dL)	5.9 ± 1.3	6.2 ± 1.4	0.342
GFR (mL/min per$1.73\,m^2$)	73.2 ± 24.7	72.3 ± 21.4	0.876
TSH (IU/mL)	1.4 (1.0–2.2)	1.4 (0.8–2.2)	0.416
Fetuin (μg/mL)	429.5 ± 134.3	376.5 ± 133.5	0.115
Spleen area (cm^2)	46.4 ± 6.1	38.1 ± 7.4	0.008
SAT (mm)	16.9 ± 6.8	13.1 ± 5.5	0.017
VAT (mm)	63.1 ± 27.8	51.7 ± 23.8	0.084
VAT/SAT ratio	4.11 ± 2.1	4.35 ± 2.1	0.664
Drugs			
Antihypertensive, *n* (%)	21 (72.4)	28 (68.3)	0.795
Lipid-lowering, *n* (%)	23 (79.3)	18 (43.9)	0.004
Hypoglycemic, *n* (%)	10 (34.5)	5 (12.2)	0.038

Data are expressed as mean ± standard deviation (SD) and median (25^{th}–75^{th} percentile) for continuous variables normally and not normally distributed as frequencies (percentages) for categorical variables.

The Student *t*-test was used for normally distributed variables. The Mann–Whitney test was used for non-normally distributed variables. The Fischer exact test was used for proportions.

NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CAD, coronary artery disease; WBCs, white blood cells; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TGs, triglycerides; HOMA, homeostasis model assessment of insulin resistance; GFR, glomerular filtration rate; TSH, thyroid-stimulating hormone; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

reporting an association between angiographically documented CAD and NAFLD, independent of other metabolic factors. More recently, Wong et al.[30] confirmed that NAFLD was an independent predictor of significant CAD (≥50% coronary stenosis) after adjusting for demographic and metabolic factors in 612 patients undergoing elective coronarography.

The chief aim of this study was to ascertain whether fetuin-A might modulate the cardiovascular risk in relation to the presence of NAFLD. In our study, multivariate analysis showed that fetuin-A predicts NAFLD independently of other metabolic factors, in agreement with recent data reported by Haukeland et al.[31] Of interest, following inclusion of the metabolic syndrome in the multivariate model, fetuin-A is no longer an independent predictor of NAFLD, suggesting that the association between NAFLD and serum fetuin-A levels is largely mediated by the presence of the metabolic syndrome.

Fetuin-A is an attractive biological mediator of atherogenesis. Given that this glycoprotein prevents ectopic calcification, a reduction of fetuin-A concentrations might, in principle, promote cardiovascular calcifications.[8] Despite these theoretical considerations, the role of fetuin-A as a cardiovascular risk factor remains controversial, with some studies suggesting that low serum fetuin-A levels are independently correlated with CAD and coronary deaths[11–13] and others reporting that high plasma fetuin-A levels are associated with increased risk of cardiovascular events.[14,15]

Multivariate analysis in the present study has shown fetuin-A to be an independent predictor of CAD in the entire study population. Interestingly, we found fetuin-A negatively correlated with WBCs, suggesting that decreased fetuin-A levels might be associated with atherogenesis via increased subclinical inflammation. Being speculative, such a view needs to

Table 3. Independent Predictors of CAD at Multivariate Analysis

	B	*SE*	*OR*	*95% CI*	P
Age	0.015	0.028	1.016	0.962–1.072	0.579
Female gender	−1.118	0.682	0.327	0.086–1.244	0.101
NAFLD	0.908	0.645	2.478	0.700–8.778	0.160
Fetuin-A	−0.005	0.002	0.995	0.991–1.000	0.049
Constant	1.507	2.146	4.512		0.483

Fixed covariates were gender, age, and NAFLD.
The overall discriminant power of the model was 72.1%.
CAD, coronary artery disease; SE, standard error; OR, odds ratio; CI, confidence interval; NAFLD, nonalcoholic fatty liver disease.

be validated by assessing high sensitivity C-reactive protein (hsCRP) and the pro-inflammatory [interleukin-6 (IL-6), IL-1B, tumor necrosis factor-α (TNF-α)] and anti-inflammatory (adiponectin) cytokine profile in future studies.

Fetuin-A is deemed to exert anti-inflammatory activities in humans.[32] A negative correlation between fetuin-A and WBCs, erythrocyte sedimentation rate, and hsCRP has been reported in patients with acute coronary syndrome.[33] Moreover, fetuin-A levels are inversely associated with disease severity and exacerbation frequency in patients with chronic obstructive lung disease.[34] However, the relationship between fetuin-A and inflammation is likely to be more complex than that; recent experimental studies have shown that fetuin-A may act as a mediator of proinflammatory responses via Toll-like receptor 4.[35]

In our study, regarding different fetuin-A serum concentrations, patients with and without CAD had different VAT thicknesses and VAT/SAT ratios. VAT has emerged as a key organ contributing to the development of CAD, and increased visceral adiposity is significantly associated with the severity of CAD, even in subjects without central obesity.[36] We found no difference in the prevalence of classical CV risk factor (age, smoking, dyslipidemia, hypertension) in patients with/without CAD. A reason may be the limited number of patients recruited. Moreover, although the prevalence of dyslipidemia was not different between CAD-positive and CAD-positive patients, the percentage of those taking lipid-lowering drugs was higher in CAD group. This finding indirectly suggests that patients eligible for drug treatment, and thus with higher global CV risk score, were included, as expected, in the CAD-positive group.

Our study has some limitations. First, given that fetuin-A is a calcification inhibitor, there was a failure to assess coronary artery calcifications. Second, the limited study sample size might have impaired the statistical power of analysis. However, despite this, we were able to show statistically significant data supporting a strong association between fetuin-A and NAFLD and CAD. Third, the lack of liver biopsy, which is the gold standard for the diagnosis of NAFLD and can differentiate simple steatosis from NASH.[1] However, liver biopsy is an invasive procedure not devoid of complications and should be performed in clinical practice only in selected cases.[1] Finally, given the cross-sectional nature of this study, we have no prospective follow-up data of changes in fetuin-A levels and CAD events in NAFLD patients whose fetuin-A was measured at baseline.

To sum up, we were able to show that increased fetuin-A levels are an independent predictor of NAFLD. On the other hand, decreased fetuin-A levels are associated with a higher risk of CAD diagnosed at elective coronarography. Given that NAFLD is almost universally deemed to be a cardiovascular risk factor,[4,30,37] it remains unclear how we move from NAFLD, a state featuring elevated fetuin-A levels, through CAD, which on the basis of our findings displays decreased fetuin-A levels. Our cross-sectional analysis could not address such key issues. Thus, prospective follow-up data obtained by recruiting a larger patient population with biopsy-proven liver disease will be necessary to answer the question of whether it is steatosis or NASH that is more atherogenic in the coronary tree.

Table 4. Independent Predictors of NAFLD at Multivariate Analysis

	B	*SE*	*OR*	*95% CI*	P
A. Model 1: Age, Gender, and BMI as fixed covariates[a]					
Age	−0.037	0.027	0.963	0.914–1.016	0.168
Female gender	0.523	0.682	1.687	0.444–6.415	0.443
BMI	0.261	0.092	1.298	1.084–1.553	0.005
Fetuin-A	0.005	0.003	1.005	1.000–1.010	0.036
Constant	−7.045	3.764	0.001		0.061
B. Model 2: Age, gender, and metabolic syndrome as fixed covariates[b]					
Age	−0.050	0.026	0.951	0.905–1.000	0.052
Female gender	0.368	0.651	1.445	0.403–5.175	0.572
Metabolic syndrome	1.269	0.553	3.556	1.202–10.519	0.022
Constant	3.080	1.797	21.751		0.087

[a]Overall discriminant power of the model is 74.6%.
[b]Overall discriminant power of the model is 65.7%. Fetuin-A is not kept in the model.
SE, standard error; OR, odds ratio; CI, confidence interval; BMI, body mass index.

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Author Disclosure Statement

The authors have no conflicts to disclose.

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