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Tumor necrosis factor-α is a marker of familial combined hyperlipidemia, independently of metabolic syndrome

Paolo Pauciullo*, Marco Gentile, Gennaro Marotta, Angela Baiano, Stefania Ubaldi, Fabrizio Jossa, Gabriella Iannuzzo, Fulvio Faccenda, Salvatore Panico, Paolo Rubba

Department of Clinical and Experimental Medicine, University "Federico II" Medical School, 80131 Naples, Italy

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Abstract

It is unclear whether an association between familial combined hyperlipidemia (FCHL) and inflammatory markers exists, independently of age, sex, body weight, insulin resistance, and metabolic syndrome. Serum concentrations of soluble vascular cell adhesion molecule-1 (sVCAM-1), monocyte chemoattractant protein 1, interleukin 6, tumor necrosis factor-α (TNF-α), and high-sensitive C-reactive protein were determined in 135 probands with FCHL and in 146 normolipidemic, normotensive, normoglycemic healthy subjects. Insulin resistance was evaluated using homeostasis model assessment (HOMA). All inflammatory parameters, except interleukin 6, were significantly higher in FCHL according to medians or mean comparisons. After adjustment for age, sex, body mass index, and HOMA, only TNF-α remained an independent predictor of FCHL status by binary logistic regression (odds ratio [OR], 1.19; 95% confidence interval [CI], 1.07-1.31; $P = .001$). In particular, elevated levels of TNF-α (above the 90th and 95th percentiles of the value observed in the control group, 9.6 and 9.8 pg/mL, respectively) were independent predictors of FCHL status: for TNF-α above the 90th percentile, OR was 7.91 (95% CI, 3.27-19.13; $P < .001$), and for TNF-α above 95th percentile, OR was 13.08 (95% CI, 4.60-37.15; $P < .0001$). The independent role of TNF-α as predictor of FCHL status was confirmed after adjustment for components of the metabolic syndrome ($P = .007$ and $P = .003$, for TNF-α values above 90th and 95th percentiles, respectively). In conclusion, among the inflammatory markers most commonly measured, only TNF-α was associated with FCHL independently of age, sex, body mass index, and HOMA. The association of TNF-α with FCHL was also independent of the metabolic syndrome.

1. Introduction

Familial combined hyperlipidemia (FCHL) and metabolic syndrome (MS) are complex metabolic disorders associated with increased cardiovascular disease (CVD) risk [1-3]. Familial combined hyperlipidemia and MS share several metabolic abnormalities, including central obesity, hypertension, insulin resistance, and hypertriglyceridemic dyslipidemia [4-7].

Robust evidence links systemic low-grade inflammation —as expressed by moderately elevated serum levels of soluble vascular cell adhesion molecule-1 (sVCAM-1), monocyte chemoattractant protein 1 (MCP-1), interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), and high-sensitive C-reactive protein (hs-CRP)—with central obesity and with MS [8-12]. Cellular adhesion molecules and MCP-1 can be produced by endothelial cells and also by adipocytes and macrophages in adipose tissue [13]; they are correlated with waist-hip ratio and body mass index (BMI) [8,10] and can be lowered by reduction of visceral fat [14]. Interleukin 6 and TNF-α are released from macrophages in the vessel wall but also from macrophages in adipose tissue and from adipose cells [14]. These cytokines decrease after weight loss and rosiglitazone treatment, as does hs-CRP—one of the hepatic-derived acute-phase reactants stimulated by vascular inflammation [14-16].

All the above inflammatory markers have been independently associated with increased CVD risk [17,18].

Less information is available on the components of this inflammatory constellation in their relationship with FCHL, independently of the metabolic abnormalities shared with MS, which is often a feature of patients with FCHL.

* Corresponding author. Tel.: +39 081 7462303; fax: +39 081 5466152.
E-mail address: pauciull@unina.it (P. Pauciullo).

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The aims of the present study were (*a*) to assess whether and which inflammatory markers are increased in patients with FCHL compared to control subjects and (*b*) to assess whether low-grade inflammation is a marker of FCHL, independently of BMI, insulin resistance, and the components of MS.

2. Patients

One hundred thirty-five patients with FCHL (30% women), among those consecutively admitted to the outpatient Lipid Clinic of the "Federico II" University of Naples, were enrolled in the study, after excluding patients with secondary causes of dyslipidemia or those using any drug known to affect lipid metabolism. Each patient was a proband from a different family.

Familial combined hyperlipidemia was diagnosed according to the criteria suggested by an Italian expert panel [19]: serum triglyceride level of greater than 200 mg/dL and/or apolipoprotein B of greater than 130 mg/dL and/or low-density lipoprotein cholesterol (LDL-C) of greater than 160 mg/dL in the proband, plus phenotype variability in at least one first-degree relative, and/or a history of premature CVD in the proband and/or in first-degree relatives.

According to the American Heart Association statement [2], and with the exclusion of patients on lipid-lowering drugs, MS was diagnosed when 3 or more of the following criteria were present:

1. Elevated waist circumference: ≥102 cm in men, ≥88 cm in women;
2. Elevated triglycerides: ≥ 150 mg/dL;
3. Reduced high-density lipoprotein cholesterol: <40mg/dL in men, <50 mg/dL in women;
4. Elevated blood pressure: systolic ≥130 mm Hg or diastolic ≥85 mm Hg; drug treatment of hypertension.
5. Elevated fasting glucose: ≥100 mg/dL; drug treatment of elevated glucose.

Eleven patients with FCHL had type 2 diabetes mellitus. Ten of them were on oral hypoglycemic agents. None of them was on insulin.

Control subjects were drawn from the registries of 3 different general practitioners in the Naples province. Two hundred clinically healthy subjects (50% women; age range, 20-65 years) with no history of metabolic, cardiovascular, or chronic diseases were invited for a clinical and biochemical checkup: acceptance rate was 85%. Inclusion criteria for control subjects were blood pressure of less than 140/90 mm Hg and LDL-C and triglyceride levels of less than 160 and 200 mg/dL, respectively. One hundred forty-six subjects (58% women) met these criteria.

By the time of the blood sampling, none of the study participants reported or showed inflammatory or other concurrent diseases.

All participants signed an informed consent, and the protocol of the study was approved by the ethics board of the "Federico II" University.

3. Methods

Blood was drawn by puncture of antecubital vein after a 12-hour fast and withdrawal for at least 5 weeks from any lipid-lowering treatment.

Total cholesterol and triglyceride concentrations were measured using standard enzymatic methods [20,21]. High-density lipoprotein cholesterol was measured after the precipitation of very-low-density lipoproteins and low-density lipoproteins with phosphotungstic acid [22], and LDL-C was calculated according to the Friedewald formula whenever serum triglyceride levels were lower than 400 mg/dL. In patients with serum triglyceride level of greater than 400 mg/dL (n = 20), LDL-C was not calculated.

Fasting glucose levels were enzymatically determined by the peroxidase method.

Fasting insulin levels were determined by enzyme immunoassay (Ultrasensitive Insulin Elisa, Mercodia, Sweden). The error of the method was evaluated on 2 sera at small and large concentrations of insulin and was less than 10%. The homeostasis model assessment (HOMA) index was used to estimate insulin resistance and calculated as fasting serum insulin (μU/mL) × fasting serum glucose (mmol/L)/22.5.

Apolipoprotein B and hs-CRP were measured by turbidimetric assay with an automated method (Cobas-Mira, Roche, Italy). The error of the method, evaluated by daily analysis of a plasma pool, was less than 5%.

Serum levels of sVCAM-1, MCP-1, IL-6, and TNF-α were measured using 3 different commercial enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN). These assays use the quantitative sandwich enzyme-immunoassay technique. The error of the methods was evaluated by repeat analysis of a plasma pool and was less than 10% [23].

Blood pressure was measured by a conventional sphygmomanometer; diastolic pressure was measured at the fifth Korotkoff tone.

The cardiovascular event categorical variable was regarded as positive when the patient or a first-degree relative had experienced myocardial infarction, stable or unstable angina, stroke, transitory ischemic attack, or peripheral vascular disease.

Patients and control subjects were regarded as smokers if presently smoking at least 5 cigarettes per day or had quit within the previous 12 months.

3.1. Statistical analysis

Data on inflammatory cytokines, triglycerides, and HOMA were nonnormally distributed variables and therefore underwent logarithmic transformation. Normal distribution was achieved only for MCP-1, triglycerides, and HOMA

Table 1
Clinical and biochemical characteristics of the study population

Variable	Control subjects (n = 146)	FCHL (n = 135)	P
% Women	58	30	<.001
Age (y)	43.2 ± 10.3	46.3 ± 12.5	<.05
Total cholesterol (mg/dL)	185.2 ± 27.3	285.1 ± 66.4	<.001
Triglycerides (mg/dL)	85.2 ± 40.0	255.4 ± 186.3	<.001
High-density lipoprotein cholesterol (mg/dL)	51.1 ± 12.5	39.2 ± 10.6	<.001
LDL-C (mg/dL)	117.1 ± 24.1	200.2 ± 62.6	<.001
Fasting glucose (mg/dL)	93.8 ± 10.8	102.2 ± 21.2	<.001
Apolipoprotein B (mg/dL)	88.0 ± 10.0	140.0 ± 30.0	<.001
Body mass index (kg/m^2)	25.2 ± 3.9	27.4 ± 4.5	<.001
HOMA index	1.3 ± 0.7	1.9 ± 1.2	<.001
Systolic blood pressure (mm Hg)	118.0 ± 16.3	135.4 ± 16.9	<.001
Diastolic blood pressure (mm Hg)	76.2 ± 10.0	85.5 ± 8.4	<.001
Smokers (%)	38	34	.571

Values are expressed as mean ± SD. International System conversion factors: to convert triglycerides to millimoles per liter, multiply by 0.0113; to convert high-density lipoprotein cholesterol to millimoles per liter, multiply by 0.02586; to convert glucose to millimoles per liter, multiply by 0.05551; to convert total and LDL-C to millimoles per liter, multiply by 0.02586.

(Shapiro-Wilk test). Unpaired *t* test was used for the comparison of normally distributed variables. The Mann-Whitney test was used for the comparison of all the inflammatory markers that were nonnormally distributed. Because of the planned several comparisons between groups, the level of significance for those tests was adjusted downward. The accepted level was .01, obtained dividing .05 by the number of comparisons, that is 5. χ^2 Statistic was used to compare the distribution of categorical variables between groups. Binary logistic regression was used to estimate the predictors (independent variables) of FCHL status (dichotomized, dependent variable). All inflammatory markers were first entered as continuous variables in unadjusted and adjusted (for age, sex, BMI, and HOMA) logistic regression. In subsequent analyses, because the distribution of TNF-α was positively skewed (skewness = 6.969), this parameter was entered in logistic analyses as a dichotomized variable: above or below the 50th, 75th, 90th, and 95th percentiles of the distribution in the control group. Three models were used to analyze the relationship of different levels of TNF-α with FCHL status. The first model was unadjusted. In the second model, age, sex, HOMA, and BMI were included. In the third model, all the components of MS were considered, except for serum triglyceride levels, as they were included in the diagnosis of FCHL status. However, a separate analysis was carried out including also triglycerides. Statistical analyses were carried out using the 12.0 version of SPSS (SPSS, Chicago, IL). A significance level lower than .05 was accepted.

Table 2
Inflammatory markers in control subjects and patients with FCHL

Variable	Control subjects	FCHL	P
hs-CRP (mg/L)	1.10 (0.60-2.0)	1.40 (1.00-2.60)	.001 [a]
IL-6 (pg/mL)	2.30 (1.70-4.40)	2.20 (1.80-2.90)	.628 [a]
sVCAM-1 (ng/mL)	412.0 (271.0-627.0)	525.0 (418.0-676.0)	<.001 [a]
MCP-1 (pg/mL)	288.0 (221.0-423.0)	360.0 (279.0-441.0)	.037 [b]
TNF-α (pg/mL)	8.30 (6.70-8.90)	8.5 (6.30-11.30)	.003 [a]

Values are expressed as median (interquartile range).
[a] Mann-Whitney.
[b] Unpaired *t* test.

4. Results

Demographic, clinical, and biochemical characteristics of the control subjects and patients with FCHL are shown in Table 1. Besides the selection-related differences in blood lipids and blood pressure, the percentage of women, age, BMI, and HOMA were significantly lower in the control group compared to patients with FCHL. In the FCHL group, 62% of the patients reported a personal or familial CVD event.

Table 2 shows serum concentrations of the inflammatory markers studied in the 2 groups. Except for IL-6 and MCP-1, all the others were significantly higher in patients with FCHL than in control subjects at the accepted .01 level of probability.

Table 3 summarizes the relationship between the inflammatory markers, entered as continuous variables, with FCHL status. Binary logistic regression is presented, both unadjusted and adjusted for age, sex, BMI, and HOMA. Among the adjusted analyses of the inflammatory markers, only TNF-α remained an independent predictor of FCHL status (odds ratio [OR], 1.19; 95% confidence interval [CI], 1.07-1.31; $P = .001$). Further adjustment for presence or absence of diabetes mellitus did not change the level of statistical significance (OR, 1.18; 95% CI, 1.07-1.30; $P = .001$).

Table 4 shows the same unadjusted and adjusted relationships using as independent dichotomized variables the 50th, 75th, 90th, and 95th percentiles of the TNF-α values observed

Table 3
Relationship of inflammatory markers, expressed as continuous variables, with FCHL status (dependent variable) (binary logistic regressions, unadjusted and adjusted for sex, age, BMI, and HOMA; FCHL n = 135, control subjects n = 146)

Independent variables	OR	95% CI	P
hs-CRP			
Unadjusted	1.09	0.98-1.21	.101
Adjusted	1.07	0.96-1.19	.228
IL-6			
Unadjusted	0.91	0.83-1.00	.044
Adjusted	0.90	0.81-1.00	.057
sVCAM-1			
Unadjusted	1.00	1.00-1.01	.055
Adjusted	1.00	1.00-1.01	.056
MCP-1			
Unadjusted	1.00	1.00-1.01	.112
Adjusted	1.00	1.00-1.01	.898
TNF-α			
Unadjusted	1.20	1.09-1.32	<.0001
Adjusted	1.19	1.07-1.31	.001

Table 4
Crude, adjusted, and MS components adjusted ORs for risk of FCHL according to plasma concentrations of TNF-α above the 50th, 75th, 90th, and 95th percentile cut points of control group distribution (binary logistic regression, dependent variable FCHL status; FCHL n = 135, control subjects n = 146)

	TNF- α	Unadjusted			Adjusted [a]			Adjusted for components of MS [b]		
	pg/mL	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
50th	>8.3	1.46	0.87-2.47	.148	1.28	0.71-2.30	.406	0.77	0.29-2.01	.591
75th	>8.9	2.56	1.46-4.50	<.001	2.28	1.21-4.30	.010	1.30	0.47-3.63	.609
90th	>9.6	7.98	3.55-17.99	<.0001	7.91	3.27-19.13	<.0001	8.03	1.75-36.86	.007
95th	>9.8	13.14	4.96-34.75	<.0001	13.08	4.60-37.15	<.0001	13.4	2.35-77.02	.003

[a] Age, sex, BMI, HOMA.
[b] Age, sex, plasma glucose, plasma high-density lipoprotein cholesterol, systolic blood pressure, diastolic blood pressure, waist circumference; triglycerides not included because these are within the definition of the dependent variable (see Results section for analysis including triglycerides).

in the control group (8.3, 8.9, 9.6, and 9.8 pg/mL, respectively). Only the adjusted relationships of the 75th, 90th, and 95th percentiles of TNF-α proved statistically significant (P =.010, P < .0001, and P < .0001, respectively).

Adjustments for CVD events did not substantially change the significance of this relationship for the 90th and 95th percentiles of TNF-α (for TNF-α 90th percentile: OR, 5.73; 95% CI, 2.03-16.23; P =.001; for TNF-α 95th percentile: OR, 10.02; 95% CI, 2.99-33.60; P < .0001).

Age-, BMI-, and HOMA-adjusted logistic regressions, carried out separately in men and in women, showed independent relationships between TNF-α and FCHL status in both sexes, although more markedly in men. For the 90th percentile of TNF-α, OR was 11.01 (95% CI, 23.07-39.56; P < .0001) in men and OR was 5.59 (95% CI, 1.41-22.20; P = .014) in women. For the 95th percentile of TNF-α,OR is 33.42 (95% CI, 4.31-259.11; P = .001) in men and OR is 7.19 (95% CI, 1.65-31.35; P = .009) in women.

In patients with FCHL with (n = 94) and without (n = 41) MS, TNF-α levels were not significantly different (median [interquartile range]): 8.60 (6.20-12.90) pg/mL vs 7.90 (6.20-11.20) pg/mL, respectively (P = .384). The influence of the components of MS on the age- and sex-adjusted relationship between TNF-α and FCHL status was evaluated (Table 4). Again, high levels of TNF-α were independent predictors of FCHL status (P = .007 for the 90th percentile of TNF-α, and P = .003 for the 95th percentile). Triglyceride levels, also a component of the MS, were not included as independent variable in the analysis shown in Table 4 because it is a determinant of the dependent variable (FCHL status). However, the significant relationships between high levels of TNF-α and FCHL status hold after adding triglyceride levels in the same model as independent variable (for the 90th percentile of TNF-α: OR, 8.47; 95% CI, 1.36-52.73; P = .022; for the 95th percentile of TNF-α: OR, 11.65; 95% CI, 1.68-80.55; P = .013).

5. Discussion

The present study addresses the issue of low-grade inflammation levels in FCHL, and their role as markers of the disease, independently of concurrent MS.

To avoid the possible lack of independence of several patients with FCHL belonging to few families, only the probands consecutively admitted to the university lipid clinic were included in the study. Because there was no sex difference in the affected relatives of the FCHL probands, a possible explanation for the significantly higher prevalence of men in our sample is a self-selection because of their own awareness of being at higher CVD risk than women.

In the unadjusted analysis, all but one of the inflammatory markers examined were significantly increased in patients with FCHL.

Interleukin 6 was about the same in the 2 groups, a finding that is in agreement with recent data on human obese subjects [10].

Because the other inflammation markers measured in the present study show a significant relationship with body fat, all comparisons were then adjusted not only for sex and age but also for BMI and HOMA, the latter as estimate of insulin resistance.

After this adjustment, serum levels of hs-CRP, IL-6, VCAM-1, and MCP-1 did not show a significant relationship with the FCHL status.

The main finding of this study was that TNF-α concentration remained significantly and independently related to FCHL status.

We investigated which levels of serum TNF-α were significantly associated with FCHL. No specific level had been suggested in previous studies as related to FCHL or other atherogenic diseases. Because the distribution of TNF-α is positively skewed, the 50th, 75th, 90th, and 95th percentiles observed in the control population, which was free of CVD risk factors, were chosen as population-based cutoff levels. Tumor necrosis factor α levels above 90th and 95th percentiles (9.6 and 9.8 pg/mL, respectively) were significantly associated with FCHL status in all adjusted logistic regressions.

Tumor necrosis factor α is a cytokine that is secreted within the endothelial wall but also in adipose tissue by adipose cells and macrophages [13]. Its relationship with obesity and obesity-related parameters was demonstrated in observational [10] and intervention studies based on weight reduction [24]. In addition, the G-308A promoter polymorphism of the TNF-α gene has been associated with MS

[25]. These data indicate the rationale behind the adjustment of the relationship between TNF-α and FCHL for the components of MS. In our sample, this relationship proved statistically significant after adjustment not only for BMI and HOMA but also for the components of MS.

The independent relationship of TNF-α with FCHL suggests a significant association between TNF-α and FCHL. In 2 articles relevant to this issue [26,27], a linkage was suggested between FCHL and the locus 1q21-23 including the TNF superfamily member-1B. Reduced plasma levels of soluble-TNF-receptor p75, 1 of the 2 binding domains of the TNF-α receptor, were found in patients with FCHL [26], thus suggesting an increased number of TNF-α receptors bound to target cells. The proposed link with the disease was an increased cellular sensitivity to the proinflammatory activity of TNF-α caused by an increased number of cell-bound, active TNF-α receptors [27]. Only in 1 of these 2 articles were TNF-α concentrations elevated in patients with FCHL compared to control subjects [27]. However, the difference was significant only with nonaffected relatives but not with normolipidemic control subjects. A possible explanation for the more pronounced differences observed in our study compared with the one by Cavallo et al [27] could be the larger patient population in the present study and the lower sensitivity of previously available methods for the measurement of TNF-α (lowest sensitivity limit of 7 pg/mL in the study by Cavallo et al, 0.5 pg/mL in ours).

It is reasonable to hypothesize that the small, dense, and more oxidizable LDL particles, typical of FCHL, could be one of the causes of increased TNF-α levels. There is actually evidence indicating that advanced oxidation protein products accelerate atherosclerosis by promoting inflammation. In animal models, plasma levels of these products, which were shown to increase aortic plaque area and macrophage infiltration, were positively correlated with TNF-α levels [28]. The lack of an antiatherosclerotic effect of antioxidant agents in humans [29] might be just due to their inability to stop inflammation once it starts. Moreover, small, dense LDLs show an enhanced interaction with the scavenger receptors of macrophages, which are responsible for almost all TNF-α expression in adipose tissue [30].

In conclusion, the present study indicates that, among inflammatory markers most commonly measured, TNF-α levels are related to FCHL status, independently of age, sex, BMI, and HOMA. The relationship of elevated TNF-α levels and FCHL was confirmed after adjustment for the components of MS. Given the available evidence that TNF-α is an independent marker of CVD, we suggest that a high level of this cytokine could identify patients with FCHL at particularly increased cardiovascular risk.

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