

Decreased Serum Lipoprotein Levels as a Guide for Clinical Severity in Patients with Idiopathic Dilated Cardiomyopathy

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SEZGIN, N., SEZGIN, A.T., GULLU, H., KARABULUT, A., BARUTCU, I., TOPAL, E., YALCINTAS, D. and TEMEL, I. *Decreased Serum Lipoprotein Levels as a Guide for Clinical Severity in Patients with Idiopathic Dilated Cardiomyopathy.* Tohoku J. Exp. Med., 2005, **206** (3), 219-224 — Hyperlipidemia is a cardiovascular risk factor. In patients with idiopathic dilated cardiomyopathy (IDC), prognostic roles of endogenous lipoproteins are not fully clarified. It has been known that there is a direct relationship between the levels of cytokines (tumor necrosis factor- α [TNF- α] and interleukin-6 [IL-6]) and deteriorating functional classes of heart failure and mortality. The present study compared the levels of circulating TNF- α , IL-6, lipoproteins, and apolipoproteins in patients with stable IDC ($n = 28$) with those of patients with unstable IDC ($n = 26$) and controls ($n = 24$). Mean serum total cholesterol (TC) was significantly lower in stable IDC patients than controls ($p < 0.05$). In unstable IDC patients, mean serum TC was also lower than controls but not statistically significant. The IDC patients had significantly higher concentrations of IL-6 and TNF- α than the controls ($p < 0.01$). Serum IL-6 and Apo AI levels were significantly different between stable and unstable IDC patients ($p = 0.021$ and $p = 0.012$, respectively). Increased levels of IL-6 were associated with decreased levels of TC ($r = -0.266$, $p = 0.019$), LDL-C ($r = -0.376$, $p = 0.001$) and apolipoprotein AI (apo AI) ($r = -0.495$, $p < 0.001$) in all IDC patients. TNF- α was also inversely related to apo AI ($r = -0.455$, $p < 0.001$) and LDL-C ($r = -0.364$, $p = 0.001$) in all patients. Thus, elevated serum levels of cytokines in patients with IDC are associated with decreased lipoprotein concentrations, which may indicate impaired prognosis. — heart failure; lipoprotein; apolipoprotein; cytokine

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Hypercholesterolemia is a well-established risk factor for the development of coronary artery disease (CAD) and CAD mortality (Pekkanen et al. 1990). Elevated serum cholesterol levels are

also risk factors for the development of heart failure (HF) (Kannel and Belanger 1991). It would be reasonable to expect that high cholesterol would be a risk factor and have deleterious effects

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on mortality in patients with established HF. However, preliminary reports have suggested that there is an increased mortality in HF patients with low cholesterol (Richartz et al. 1998; Vredevoe et al. 1998; Rauchhaus et al. 2000).

Cytokines, which are polypeptides produced by cells of the immune system and of some tissues, act as mediators of the immune and acute-phase responses (Mimasaka et al. 2001). Tumor necrosis factor- α (TNF- α) and other proinflammatory cytokine parameters can be elevated in patients with advanced coronary HF (Levine et al. 1990; Anker et al. 1997). It has been reported that higher cholesterol levels were associated with lower levels of TNF- α (Vredevoe et al. 1998).

In this study we aimed to evaluate the relationship between serum lipids including triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), apolipoprotein AI, B (apo AI, B) concentrations and circulating serum concentrations of cytokines including tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) in patients with idiopathic dilated cardiomyopathy (IDC).

MATERIALS AND METHODS

Patients

We prospectively studied 78 consecutive patients referred to our hospital for diagnostic procedures. Inclusion criteria for patients with IDC were: 1) no evidence of coronary artery disease as assessed by coronary angiography and 2) global moderate-severe left ventricular dysfunction (global hypokinesis). Left ventricular ejection fraction was determined by echocardiography and left ventricular cineangiography. Fifty-four patients with IDC were enrolled in the study. Then, IDC patients were separated according to their New York Heart Association (NYHA) class; 28 patients were in clinically stable condition without signs of pulmonary edema, NYHA functional classes I-II (stable IDC group), and the remaining 26 patients had signs of heart failure (dyspnea, pulmonary edema, on chest x-ray), NYHA III-IV (unstable IDC group). Twenty-four patients who did not have clinical, echocardiographic, and angiographic evidence of heart disease served as the control group. Exclusion criteria for both IDC patients and normal controls were: 1) causes of cardiomyopathy (other than IDC for the pa-

tients), such as valvular, restrictive, or hypertrophic, or due to sarcoidosis, amyloidosis, hemochromatosis, pericardial, and/or congenital heart disease; 2) diabetes mellitus or other systemic disease (malignancy, collagen vascular disease); 3) previous cardiac surgery; 4) elevated serum creatinine (> 2.5 mg/100 ml); and 5) pregnancy. This study was approved by the Ethical Committee of Inonu University School of Medicine, and all participants gave written, informed consent.

Methods

Blood samples were taken in the morning after 12-hour overnight fasting. Serum glucose, total cholesterol and triglycerides were determined by enzymatic colorimetric assays (Olympus AU 600, Tokyo) with coefficient of variations (CV) at $< 5.5\%$. HDL cholesterol was determined by a homogeneous assay based on polyanionic synthetic polymers and detergents (Olympus AU 600) with a CV of 3.4%. This method meets the goals of the 1998 NIH National Cholesterol Education Program for acceptable performance. The results of the method correlate well with those obtained by precipitation-based methods (Second report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults [Adult Treatment Panel II], September 1993). LDL cholesterol levels were determined by the Friedewald formula (Friedewald et al. 1972). Apo AI and B levels were determined by immunonephelometry (Behring Nephelometer BN-100, Dade Behring Marburg, Germany). The analytic CV's were $< 4\%$ for both apo AI and apo B measurements. IL-6 and TNF- α were measured by enzyme linked chemiluminescence assay with Immulite analyzer (Diagnostic Product Corporation, Los Angeles, CA, USA), and the CV's for these tests were $< 6\%$. The assays were calibrated by the commercial standards of the manufacturers.

Statistics

Data sets were analyzed with SPSS software (Statistical Package for the Social Sciences, version 11.00, SSPS Inc, Chicago, IL, USA) and are presented as means \pm s.d. Differences between groups were analyzed with one-way ANOVA and Pearson's correlation was used to analyze biochemical variables. *P* values < 0.05 were considered as significant.

RESULTS

Table 1 lists the characteristics of the patients with IDC (stable and unstable) and controls.

Table 1. Parameter means and the examination of the difference of groups

		Control (n = 24)	Stable IDC group (n = 28)	Unstable IDC group (n = 26)	p-I	p-II	p-III
Age	Year	49.1 ± 10.48	56.00 ± 8.98	60.53 ± 1.69			
M/F		13/11	15/13	15/11			
EF	%	64 ± 4	39 ± 4	25 ± 4	< 0.001*	< 0.001*	< 0.001*
Hypertension		0	4	5	0.031*	0.013*	0.627
Smoking		5	6	5	0.958	0.888	0.841
B-blocker		0	2	1	0.142	0.308	0.592
ACE-inh.		0	7	6	0.002*	0.005*	0.869
ARB		0	5	4	0.014*	0.030*	0.807
Statin Rx at presentation		0	2	2	0.142	0.141	0.939
GLU	mmol/l	5.77 ± 1.78	7.27 ± 1.78	7.66 ± 2.66	0.004*	0.004*	0.483
TG	mmol/l	2.05 ± 0.92	1.56 ± 0.72	1.55 ± 0.96	0.116	0.11	0.99
TC	mmol/l	6.02 ± 0.87	5.09 ± 1.54	5.26 ± 1.35	0.034*	0.105	0.888
HDL-C	mmol/l	1.01 ± 0.16	0.99 ± 0.19	0.92 ± 0.22	0.967	0.241	0.326
LDL-C	mmol/l	3.95 ± 0.31	3.52 ± 0.8	3.44 ± 0.98	0.113	0.056	0.928
Apo-AI	g/l	1.43 ± 0.06	1.31 ± 0.28	1.13 ± 0.23	0.109	< 0.001*	0.012*
Apo-B	g/l	1.05 ± 0.17	0.96 ± 0.25	0.93 ± 0.25	0.322	0.122	0.827
TNF- α	Pg/ml	4.48 ± 0.54	6.18 ± 2.58	6.98 ± 2.25	0.01*	< 0.001*	0.328
IL-6	Pg/ml	5.85 ± 0.98	7.8 ± 3.86	12.26 ± 9.5	0.47	0.001*	0.021*

Data are expressed as mean ± s.d., $p < 0.05$ accepted significant (*) p -I: between control and NYHA class I-II (stable IDC group), p -II: between control and NYHA class III-IV (unstable IDC group), p -III: between NYHA class I-II and NYHA class III-IV.

M/F, men/female; EF, ejection fraction; GLU, glucose; TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; Apo-AI, apoprotein AI; Apo-B, apoprotein B; TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; B-blockers, beta-blockers; ACE-inh., angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; Rx, treatment.

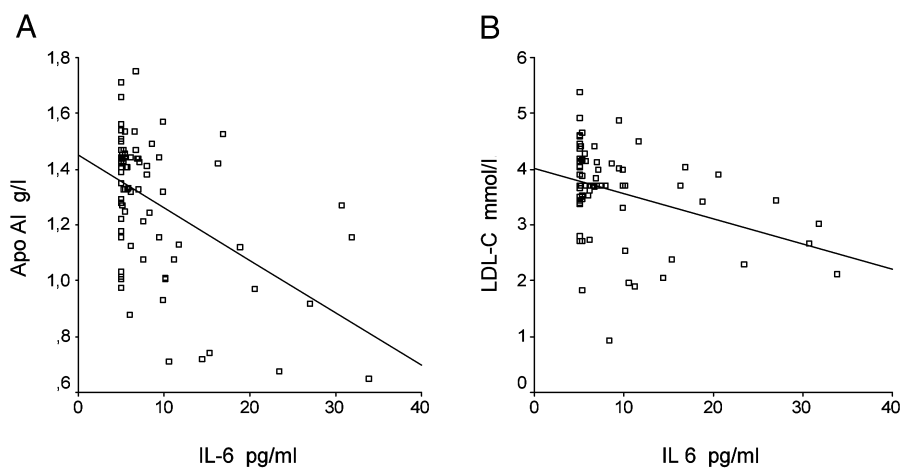


Fig. 1. Simple correlation of IL-6 with apo AI (A), and LDL-C (B) in IDC patients. There was an inverse correlation between levels of IL-6 and apo AI ($r = -0.495$, $p < 0.001$), and LDL-C ($r = -0.376$, $p = 0.001$).

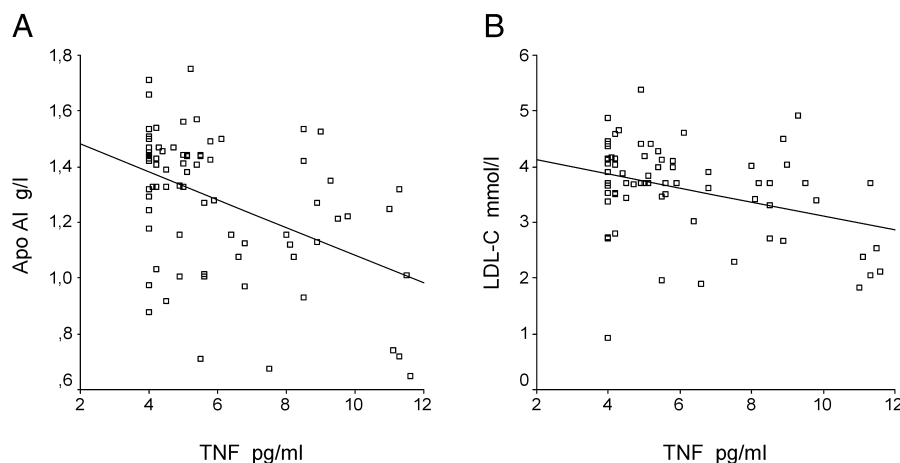


Fig. 2. Simple correlation of TNF- α with apo AI (A), and LDL-C (B). There was an inverse correlation between levels of TNF- α and apo AI ($r = -0.455$, $p < 0.001$) and LDL-C ($r = -0.364$, $p = 0.001$).

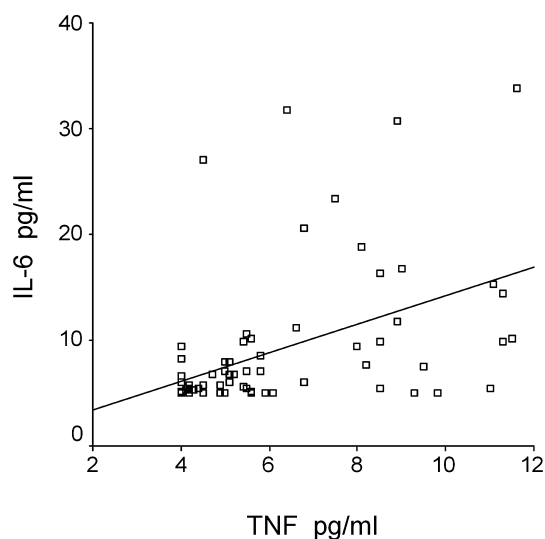


Fig. 3. Simple correlation of IL-6 with TNF- α . There was strong relationship between serum concentrations of TNF- α and IL-6 ($r = 0.472$, $p < 0.001$).

There were no significant differences between stable IDC and unstable IDC patients in baseline characteristics, including age, prevalence of diabetes and hypertension, history of prior smoking, and treatment with angiotensin-converting enzyme (ACE) inhibitors, statins. The IDC patients had significantly lower serum glucose and apo AI than the controls ($p < 0.05$). Mean serum TC was significantly lower in patients with stable IDC than the controls ($p < 0.05$). In unstable IDC

group, mean serum TC were also lower than the controls but it was not statistically significant. Mean serum TG, LDL-C, HDL-C, apo B were lower in patients with IDC than the controls but these were not statistically significant. The IDC patients had significantly higher concentrations of IL-6 and TNF- α than the controls ($p < 0.01$). Serum IL-6 and Apo AI levels were significantly different between stable and unstable IDC patients ($p = 0.021$ and $p = 0.012$, respectively). Increased serum IL-6 and decreased serum Apo AI levels were associated with clinical severity in patients with IDC. In all IDC patients, there is an inverse correlation between levels of IL-6 and lipid parameters such as total cholesterol ($r = -0.266$, $p = 0.019$), LDL-C ($r = -0.376$, $p = 0.001$) and apo AI ($r = -0.495$, $p < 0.001$) as shown in Fig. 1. Likewise, there is an inverse correlation between levels of TNF- α and lipid parameters such as apo AI ($r = -0.455$, $p < 0.001$) and LDL-C ($r = -0.364$, $p = 0.001$) as shown in Fig. 2. We found a strong relationship between serum concentrations of TNF- α and IL-6 ($r = 0.472$, $p < 0.001$) as shown in Fig. 3.

DISCUSSION

The main findings of the present study are as follows: 1) serum concentrations of IL-6 and TNF- α are increased in patients with IDC and 2) there is an inverse correlation between IL-6 and

TC, LDL-C, or apo AI in IDC patients. TNF- α is also inversely related to apo AI or LDL-C in all study groups. To our knowledge, this is the first study that has evaluated serum concentrations of apolipoproteins (apo AI and apo B) in patients with IDC.

At first sight, it may appear that cholesterol has a deleterious effect in HF. There are little data on the effects of lipoprotein levels in patients with established HF. In our study, the levels of serum TC were significantly lower in patients with NYHA class I-II than the controls ($p < 0.05$). In patients with NYHA class III-IV, the levels of TC were also lower than controls with no significant difference. Serum TG, LDL-C, HDL-C and apo B levels were lower in patients with IDC than the controls but these were also not statistically different. Our findings confirm the paradox that total cholesterol levels are decreased proportionally with the severity of HF (Richartz et al. 1998; Vredevoe et al. 1998; Rauchhaus et al. 2000). This inverse relationship between mortality and lipid lipoprotein levels has been observed in other disease states and populations (Lowrie and Lew 1990; Gui et al. 1996; Elliot and Wiles 1997; Fraunberger et al. 2000). Critical care literature provides strong evidence for a survival advantage associated with higher lipid levels in critically ill patients. Adverse outcomes associated with low serum cholesterol have been reported in trauma, surgical illness, multiple organ failure, dialysis patients, and sepsis (Lowrie and Lew 1990; Gui et al. 1996; Elliot and Wiles 1997; Fraunberger et al. 2000). Low serum cholesterol has also been associated with decreased survival rates in the elderly (Schatz et al. 2001). We believe that, with the onset of critical disease, including HF, the classic relationship between elevated cholesterol and increased mortality is no longer applicable.

The increased concentrations of cytokines (IL-6 and TNF- α) present in HF correlates with HF severity. Cytokines may contribute to HF progression and cardiac injury via their proapoptotic and negative inotropic effects (Feldman et al. 2000). IL-6 has been linked to HF severity (Torre-Amione et al. 1996; Deng et al. 1996), and it has been associated with a poor short-term

(MacGowan et al. 1997) and long-term clinical outcome (Roig et al. 1998). Rauchhaus et al. (2000) showed that a TC level < 200 mg/100 ml predicted a poor clinical outcome independently of other risk factors. In this study, higher TC levels were associated with lower levels of the cytokine TNF- α . The authors hypothesize that lipoproteins in plasma can bind and detoxify endotoxins such as lipopolysaccharide, a well-known strong stimulator for the release of inflammatory cytokines by monocytes and macrophages. This hypothesis may explain the observed relationship between lower lipoprotein levels, higher cytokine concentrations, and impaired prognosis (Rauchhaus et al. 2000).

Our study was consistent with other studies (Koller-Strametz et al. 1998; Aukrust et al. 1999) in finding that IL-6 concentrations were increased in patients with NYHA class III-IV. The correlation analysis revealed that IL-6 concentrations were negatively associated with TC ($r = -0.266, p = 0.019$), LDL-C ($r = -0.376, p = 0.001$) and apo AI ($r = -0.495, p < 0.001$). TNF- α concentrations were also negatively associated with apo AI ($r = -0.455, p < 0.001$) and LDL-C ($r = -0.364, p = 0.001$) in the present study, implying that elevated serum levels of cytokines in patients with IDC are associated with decreased lipoprotein concentrations and may indicate impaired prognosis.

Study limitation: Statistical analysis is limited by the sample size of the study.

In conclusion, the current study showed that lipoprotein levels were decreased and serum cytokine concentrations were increased in patients with IDC regarding to NYHA class. It also sees a negative association between these two concentrations. The negative relationship between lipoproteins and cytokines may indicate that low lipoprotein serves as a potential marker of poor prognosis in patients with IDC. The primary goals for the treatment of HF are improvements in survival and in quality of life. To achieve these goals, treatments need to be tailored individually for each patient. Our study indicates that the classic risk profile may not apply to patients once IDC is established.

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