Anti-Lipoprotein Lipase Antibodies in Patients with Hypertriglyceridemia without Associated Autoimmune Disease

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ABSTRACT: **Background:** Anti-lipoprotein lipase antibodies have been described in rare cases of patients with hypertriglyceridemia. However, no systematic study evaluating these antibodies in patients with this lipid abnormality has been undertaken.

Objectives: To analyze the correlation of anti-lipoprotein lipase (anti-LPL) antibodies with other laboratory findings in patients with hypertriglyceridemia but no autoimmune disease.

Methods: We evaluated 44 hypertriglyceridemic patients without autoimmune disease. Clinical and laboratory evaluations included analyses of comorbidities, fasting lipid profile and anti-LPL antibodies.

Results: Mean patient age was 55 ± 10 years; 46% of the patients were female and 64% were Caucasian. The mean disease duration was 94.4 months and mean body mass index 28.7 ± 3.6 kg/m^2; 34.0% were diabetic, 25.0% were obese, 72.7% had systemic arterial hypertension, 75% were sedentary, 15.9% were smokers, 56.8% had a family history of dyslipidemia, 45.5% had a family history of coronary insufficiency, 20.5% had acute myocardial infarction, 9.0% had undergone revascularization and 11.0% angioplasty, 79.5% were being treated with statins and 43.2% were taking fibrates. Median triglyceride levels were 254 mg/dl (range 100–3781 mg/dl), and total cholesterol level was 233 ± 111 mg/dl. High-density lipoprotein was 42.6 ± 15.4 mg/dl, low-density lipoprotein 110.7 ± 42.4 mg/dl and very low-density lipoprotein 48 ± 15 mg/dl. Anti-LPL antibodies were identified in 2 patients (4.5%), both of whom had a family history of dyslipidemia, coronary insufficiency and acute myocardial infarction; one had undergone myocardial revascularization and percutaneous transluminal coronary angioplasty, and both were using fibrates and had normal triglyceride levels.

Conclusions: Our findings demonstrate a correlation between the immune response and dyslipoproteinemia in hypertriglyceridemic patients, suggesting that autoimmune disease contributes to the dyslipidemia process.

IMAJ 2011; 13: 350–353

KEY WORDS: lipoprotein lipase, anti-lipoprotein lipase, atherosclerosis, hypertriglyceridemia, dyslipidemia

Lipoprotein lipase plays a key role in lipid metabolism by catabolizing triglycerides, which are the major components of chylomicrons and particles of very low-density lipoprotein cholesterol. Physiologically, LPL can maintain low levels of triglycerides in the circulating plasma [1]. Therefore, LPL deficiency can lead to hypertriglyceridemia, hypercholesterolemia or a combination of the two [2].

A marked decrease in LPL activity has been reported in patients with systemic lupus erythematosus [3]. This observation has led to the hypothesis that decreased LPL activity results from the inhibitory effect of an inflammatory disease or from a modulatory effect of autoantibodies. Our study group recently investigated the latter hypothesis [3]. Specifically, we showed that the incidence of anti-LPL antibodies was approximately 40% among patients with SLE. In addition, the presence of anti-LPL antibodies has been shown to positively correlate with triglyceride levels [3]. Anti-LPL antibodies have also been identified in patients with autoimmune rheumatic diseases, such as rheumatoid arthritis and systemic sclerosis [4,5], and in some case reports of patients without autoimmune diseases [6-8]. However, there have been no case studies or population studies of dyslipidemia that systematically investigated anti-LPL antibodies.

The purpose of the present study was to investigate the presence of anti-LPL antibodies and determine whether they correlate with other clinical and laboratory variables in hypertriglyceridemic patients who do not have an overt autoimmune disease.

PATIENTS AND METHODS

Forty-four patients with hypertriglyceridemia were selected from among patients treated at the Dyslipidemia Outpatient Clinic of the Instituto do Coração do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (Heart Institute of the University of São Paulo School of Medicine Hospital das Clínicas) between June 2004 and December 2004.

LPL = lipoprotein lipase
SLE = systemic lupus erythematosus

The patients were clinically evaluated and their medical charts were carefully reviewed. We excluded patients presenting clinical or laboratory evidence of autoimmune diseases such as SLE, rheumatoid arthritis, systemic sclerosis, polymyositis, dermatomyositis, mixed connective tissue disease, psoriasis and seronegative rheumatic diseases (ankylosing spondylitis, psoriatic arthritis and enteropathic arthropathies). This study was approved by the local research ethics committee. All of the patients participating in this study provided written informed consent.

LABORATORY ANALYSIS

At the study outset, 12-hour fasting blood samples were collected from the patients, all of whom were on their normal diets. All blood samples were submitted to biochemical analysis.

ASSAY DETECTING ANTI-LPL ANTIBODIES

The reactivity of anti-LPL immunoglobulin G was determined by enzyme-linked immunosorbent assay. In brief, 96-well polystyrene plates (Corning-Costar, Cambridge, MA, USA) were sensitized overnight in 5 µg/ml of commercially available LPL purified from bovine milk (Sigma, St Louis, MO). The assays were performed using serum samples diluted in Tris-buffered saline (1:100) containing adult bovine serum. Anti-LPL IgG antibodies were detected using goat anti-human IgG antibodies conjugated with alkaline phosphatase (Sigma). The reaction was developed with p-nitrophenyl phosphate, and optical density was read at a wavelength of 405 nm using a spectrophotometer (Labsystems Multiskan MS, Helsinki, Finland). Samples were considered positive if the optical density was ≥ 3 standard deviations above the mean optical density obtained for the 20 control samples from healthy individuals included in each assay [3]. Immunoblotting was used to confirm the results in samples that tested positive.

LIPID PROFILE

Total cholesterol and triglycerides were measured using enzyme immunoassays (Boehringer-Mannheim, Buenos Aires, Argentina and Merck, Darmstadt, Germany, respectively) and an RA-1000 analyzer (Technicon Instruments Corp., Tarrytown, NY, USA) [9,10]. HDL-C was quantified after VLDL and LDL-C had been precipitated using phosphotungstic acid and magnesium chloride [11]. Levels of VLDL-C and LDL-C were estimated because triglyceride levels were lower than 400 mg/dl in all samples [12]. Levels of VLDL-C were calculated by dividing triglyceride levels by 5 (TG/5) [3,4], whereas LDL-C levels were estimated using the following equation [12]:

TC = HDL + [TG/5] + LDL
where TC is total cholesterol.

STATISTICAL ANALYSIS

Descriptive statistics were used in the analysis of the results. The results are expressed as mean ± standard deviation, as median and range (for triglycerides, due to the non-Gaussian distribution of the data) or as number and percentage.

RESULTS

The mean age of the study sample was 55 ± 10 years. Of the 44 hypertriglyceridemia patients evaluated, 20 (46%) were female and 24 (54%) were male. Caucasian patients accounted for 64% of the sample group. Mean disease duration was 94.4 ± 88.2 months [Table 1].

Mean body weight was 76.7 ± 13.6 kg and mean body mass index 28.7 ± 3.6 kg/m^2. The following cardiovascular risk factors were identified: diabetes mellitus in 15 patients (34%), obesity in 11 (25%), systemic arterial hypertension in 32 (72.7%), sedentary lifestyle in 33 (75%), current smoking in 7 (15.9%) and previous history of smoking in 14 patients (31.8%). There were 25 patients (56.8%) with a family history of dyslipidemia and 20 (45.5%) with a family history of coronary insufficiency. There were also 9 patients (20.5%) with a history of acute myocardial infarction, 4 (9%) of whom had

Table 1. Demographic data, comorbidities, clinical manifestations of cardiovascular disease and medication use in 44 patients with hypertriglyceridemia

Mean age (yrs)	55 ± 10
Female	20 (46)
Caucasian	28 (64)
Disease duration (mos)	94.4 ± 88.2
Body mass index (kg/m^2)	28.7 ± 3.6
Diabetes mellitus	15 (34.0)
Systemic arterial hypertension	32 (72.7)
Sedentary lifestyle	33 (75.0)
Obesity	11 (25.0)
Smoking	7 (15.9)
Previous smoking	14 (31.8)
Family history of dyslipidemia	25 (56.8)
Family history of coronary insufficiency	20 (45.5)
History of acute myocardial infarction	9 (20.5)
History of myocardial revascularization	4 (9.0)
History of coronary angioplasty	5 (11.4)
Using fibrates	19 (43.2)
Using statins	35 (79.5)

Values are expressed as mean ± SD or as n (%)

IgG = immunoglobulin G
HDL-C = high-density lipoprotein-cholesterol
VLDL = very low-density lipoprotein
LDL-C = low-density lipoprotein

Table 2. Lipoprotein levels in 44 patients with hypertriglyceridemia

Triglycerides (mg/dl)*	254 (range 100–3781)
Total cholesterol (mg/dl)	233 ± 111
HDL-C (mg/dl)	42.6 ± 15.4
LDL-C (mg/dl)	110.7 ± 42.4
VLDL-C (mg/dl)	48 ± 15

Values are expressed as mean ± SD or as median (range)

undergone myocardial revascularization and 5 (11.4%) who had undergone angioplasty. Of the 44 patients 30 (68.2%) were being treated with statins alone, 10 (22.7%) with fibrates alone and 4 (9.1%) with a combination of the two. None of the patients had lipemia retinalis or xanthomas.

The median level of triglycerides was 254 mg/dl (range 100–3781 mg/dl). Mean levels of total, HDL, LDL and VLDL cholesterol are shown in Table 2.

The positive presence of IgG antibodies directed against LPL, as assessed by ELISA, was observed in 2 patients (4.5%). These results were confirmed by immunoblotting. It is noteworthy that both of these patients had a family history of dyslipidemia and coronary insufficiency and a personal history of acute myocardial infarction. One of these patients had undergone myocardial revascularization and percutaneous transluminal coronary angioplasty, and both patients were being treated with fibrates and had normal triglyceride levels.

DISCUSSION

The results of this study show that patients with hypertriglyceridemia and a family history of dyslipidemia or genetic defects can present anti-LPL antibodies. Because anti-LPL antibodies can be found in patients with autoimmune rheumatic diseases [3-5], only hypertriglyceridemic patients without any other autoimmune rheumatic disease were included in the present study. The target antigen for anti-LPL antibodies is the principal enzyme responsible for the hydrolysis of circulating triglycerides and facilitates plasma lipoprotein metabolism [1]. Further, decreased LPL activity is implicated in hypertriglyceridemia, which plays a key role in the atherosclerotic process [1,2].

Anti-LPL antibodies have been implicated in mechanisms of atherosclerosis in SLE and other autoimmune inflammatory diseases such as rheumatoid arthritis and systemic sclerosis [3-5]. These antibodies have been closely linked to elevated triglyceride levels in SLE and scleroderma; a putative role of anti-LPL antibodies in SLE and scleroderma has been based on the observation that the anti-LPL IgG fraction from patients with scleroderma and elevated serum triglyceride levels is capable of significantly inhibiting LPL activity in vitro [5].

The prevalence of anti-LPL antibodies in SLE, polymyositis, progressive systemic sclerosis, rheumatoid arthritis, and Sjogren's syndrome was 49 of 105 patients (46.7%), 12/30 (40%), 13/31 (41.9%), 10/90 (12.5%) and 3/30 (10%), respectively. Based on this, the authors concluded that antibodies directed against LPL were not disease-specific because they occurred as frequently in patients with polymyositis and progressive systemic sclerosis as they did in patients with SLE [4].

Recently, our group studied the presence of anti-LPL antibodies in patients with Takayasu's arteritis and its association with inflammatory markers and lipoprotein risk level. We showed that anti-LPL antibodies were not implicated in the pathophysiology of inflammatory atherosclerosis in patients with Takayasu's arteritis [13]. In addition, our group did not find any evidence of anti-LPL antibodies in Behçet's disease [14].

Regarding antiphospholipid syndrome, our group studied the traditional risk factors for coronary artery disease (i.e., homocysteine, anti-oxidized LDL, anti-LPL and endothelin) in patients with primary antiphospholipid syndrome and suggested a possible association between these variables and arterial thrombosis. Interestingly, anti-LPL antibodies were not detected in patients or controls. The authors concluded that primary antiphospholipid syndrome patients have other risk factors besides antiphospholipid antibodies that contribute to arterial events, the most important factor being hypertension [15].

Experimental studies have demonstrated that the association between autoimmune disease and LPL can lead to circulating LPL inhibitors, resulting in hyperchylomicronemia in patients with such diseases [16]. For instance, Pruneta-Deloche et al. [8] reported complete normalization of plasma triglyceride levels after immunosuppression in a patient with non-familial hyperchylomicronemia. In this case, the normalization of lipid levels coincided with an anti-LPL antibody-negative seroconversion [8].

The concept of autoimmune hyperlipidemia was introduced by Beaumont in 1970 [17,18]. However, only a few studies have described the presence of type I hyperchylomicronemia in the context of autoimmune diseases, especially in association with SLE [19-21]. One case of a patient with Graves' disease and idiopathic thrombocytopenic purpura accompanied by type I hyperchylomicronemia has been reported. In that patient, the presence of IgA antibodies to LPL was confirmed [21]. Furthermore, autoimmune hyperchylomicronemias are rare diseases that can be linked to anti-LPL antibodies. Pruneta et al. [8] described a case of a 35 year old woman suffering from severe type I hyperchylomicronemia. This patient's post-heparin plasma lipase activity showed a reduced LPL level of approximately 30% of normal. Western blot analysis revealed that the circulating anti-LPL autoantibodies were bound to chylomicrons. These data constituted the first description of

ELISA = enzyme-linked immunosorbent assay

autoimmune hyperchylomicronemia due to an exclusive loss of LPL activity. They also showed that a complete remission could be obtained in the patient following immunosuppressive therapy. Finally, the finding that anti-LPL autoantibodies were bound to chylomicrons emphasized the previously unrecognized ability of chylomicrons to transport LPL, a capability that has already been described for other lipoprotein fractions. A different study [22] investigated a patient suffering from recurrent hypertriglyceridemic pancreatitis without a family history or conventional secondary cause of dyslipidemia. These investigators identified a previously unreported heterozygous LPL gene mutation (S172fsX179) that was associated with an anti-human LPL IgG. This autoantibody partially inhibited wild-type LPL activity in vitro. Furthermore, the patient's plasma triglyceride concentrations were markedly decreased following immunosuppression and later confirmed by sequential withdrawal and reintroduction tests. The authors concluded that this unique combination of a genetic defect and an autoimmune disease resulted in chronic major hypertriglyceridemia. Because immunosuppression can improve this dyslipidemia, an assessment of the anti-LPL autoantibody level is worthwhile in unmanageable chronic major hypertriglyceridemia, even in the presence of a heterozygous LPL deficiency.

The results of the present study conclusively demonstrate that anti-LPL antibodies can play a role in the pathophysiology of hypertriglyceridemia in a subgroup of patients, including those without concomitant autoimmune disease.

Acknowledgments

This study was financially supported by the *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP, Foundation for the Support of Research in the State of São Paulo; grant no. 02/03867-0), the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, National Council for Scientific and Technological Development, grant no. 304756/2003-2 to E Bonfá, and 300665/2009-1 to JFC) and the Federico Foundation (to JF Carvalho and E Bonfá).

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"A healthy attitude is contagious but don't wait to catch it from others. Be a carrier"

Tom Stoppard (born 1937), British playwright, who has written prolifically for TV, radio, film and stage. Themes of human rights, censorship and political freedom pervade his work along with exploration of linguistics and philosophy. Stoppard has been a key playwright of the National Theatre and is one of the most internationally performed dramatists of his generation. He was knighted in 1997