ORIGINAL ARTICLE

Lack of anti-lipoprotein lipase in Behçet's disease

Jozélio Freire de Carvalho[1], Vilma Santos Trindade Viana[1], Cleonice Bueno[2], Célio Roberto Gonçalves[3], Eloísa Bonfá[4]

ABSTRACT

Objective: The recent description of anti-lipoprotein lipase antibodies (anti-LPL) associated with dislipoproteinemia led us to analyze its presence and possible association with clinical and laboratorial findings in patients with Behçet's disease. **Patients and Methods:** Thirty-eight consecutive patients with Behçet's disease [International Study Group for Behçet's Disease (ISGBD) criteria] were tested for the presence of anti-LPL antibodies by ELISA. Patients underwent clinical and laboratorial evaluation, including fasting lipid profile, determination of autoantibodies, and inflammatory markers (CRP, ESR) before inclusion in the study. Exclusion criteria were as follows: any conditions that affected the lipid profile. **Results:** Patients had a mean age of 42 ± 9 years, 68% were females, and 68% were Caucasian. Mean disease duration was 9.8 ± 7.5 years. Twenty-nine percent of the patients had a history of thrombosis. C-reactive protein levels were elevated in 31% of the patients, and ESR was increased in 31% of the patients, with mean levels of 5.95 ± 10.3 mcg/mL and 14.5 ± 13.2 mm/1st hour, respectively. Approximately 47% of the patients were taking prednisone, with a mean dose of 7.6 ± 10.8 mg/day. As for NCEP/ATPIII cardiovascular risk levels, cholesterol levels were elevated in 26% of the patients, triglycerides in 18%, low HDL in 15%, and elevated LDL in 25% of the patients with Behçet's disease. Mean total cholesterol levels were 198 ± 48 mg/dL, triglycerides 121 ± 61 mg/dL, HDL 52.4 ± 14.7 mg/dL, and LDL 119 ± 35 mf/dL. IgG anti-LPL antibodies were detected in 0/30 patients with Behçet's disease. **Conclusion:** The data presented here indicates the lack of correlation among inflammation, immune response, and dislipoproteinemia in patients with Behçet's disease and suggest**s** that other mechanisms are associated with the dyslipedemia seen in those patients.

Keywords: Behçet's disease, vasculitis, lipoprotein lipase, autoantibodies, atherosclerosis, lipoproteins, inflammation.

INTRODUCTION

Lipoprotein lipase (LPL) is a component of the lipase family, which hydrolyzes triglyceride molecules found in lipoprotein particles.[1] Physiological regulators of LPL include triglycerides and apolipoprotein (apo) CII, which increase the enzymatic activity of LPL, apo C-III, which inhibits its activity.[1]

The report of a significant reduction in LPL activity in SLE patients[2] led to the hypothesis that it resulted from an inhibitory inflammatory condition or modulation by an autoantibody. Indeed, this last hypothesis was strengthened by a recent report of an increased incidence of anti-lipoprotein lipase antibodies in SLE, which showed a positive correlation with triglyceride levels.[2,3] Those antibodies have also been reported in association with other rheumatologic disorders, including rheumatoid arthritis and systemic sclerosis.[4]

It is reasonable to speculate that the same risk factors seen in SLE patients can also be present in individuals with Behçet's disease. The objective of the present study was to evaluate the presence of anti-LPL antibodies and the possible association with clinical and laboratorial parameters of this disorder.

PATIENTS AND METHODS

Patients: Thirty-eight patients with Behçet's disease (BD) were randomly selected among those followed-up at the Behçet's Disease Outpatient Clinic of the Rheumatology

Department of Hospital das Clínicas at the Medical School at Universidade de São Paulo from June 2001 to December 2003. All patients fulfilled the International Study Group for Behçet's Disease[5] (ISGBD) criteria. Patients were clinically evaluated and their medical records were carefully reviewed. Exclusion criteria were as follows: clinical and/or laboratorial evidence of diabetes mellitus, liver and thyroid disorders, renal failure, nephritic syndrome, history of alcohol abuse, use of hypolipidemic agents, and menopause. All patients were younger than 50 years, had serum creatinine < 1.5 mg/dL, and proteinuria lower than 1.0 g/L/day.[6-8] Individuals taking medications that could interfere with the lipid profile, such as statins and fibrates, were also excluded. Female patients were not pregnant or menopausal at the time of the study. The local Ethics Committee approved the study # 814/01 and patients signed an informed consent.

Laboratorial evaluation: Serum obtained from patients after a 12-hour fasting period underwent immunological and biochemical analysis. All patients were on a regular diet.

Assay for the detection of anti-lipoprotein lipase (LPL) antibodies: The reactivity of anti-LPL IgG was measured by ELISA (Enzyme-Linked Immunosorbent Assay). Briefly, the wells of Costar® polystyrene plates were sensitized overnight with commercially available LPL from bovine milk (5 µg/mL) (Sigma Chem Co., St. Louis, MO, USA). Sera samples diluted to 1/100 in Tris buffered NS with adult bovine serum were used for the assays. IgG anti-LPL antibodies were detected with anti-human IgG goat antibodies conjugated with alkaline phosphatase (Sigma Chem Co., St. Louis, MO, USA). The reaction was developed with p-nitrophenylphosphate and the optical density (OD) was read at a 405 nm wavelength be a Labsystems Multiskan MS spectrophotometer (Helsinki, Finland). Positive results were defined as values ≥ 3 standard deviation (SD) above the mean OD of the serum samples from 20 healthy individuals included in each assay.[2]

Lipid profile: Total cholesterol (TC) and triglyceride (TG) levels were measured enzymatically (Boehringer-Mannhein, Argentina, and Merck, Germany, respectively) in a RA 1000 Analyzer (Technicon Instruments Corp.).[9,10] High-density lipoprotein cholesterol (HDL-c) was obtained after precipitation of very low density lipoprotein cholesterol (VLDL-c), and low density lipoprotein cholesterol (LDL-c) using phosphotungstic acid and magnesium chloride.[11] Very low density lipoprotein cholesterol and LDL-c were estimated, since triglyceride (TG) levels in all samples were below 400 mg/dL.[12] The levels of VLDL-c were calculated using the triglyceride levels/5 (TG/5)(34) correlation, and LDL-c levels were estimated using the following equation:[12] TC =

HDL-c+TG/5+LDL-c. Cardiovascular risk was determined according to the criteria of the National Cholesterol Education Program – Adult Treatment Panel III (NCEP/ATPIII), as follows: TC greater than 200 mg/dL, TG greater than 150 mg/dL, HDL-c lower than 40 mg/dL, and LDL-c greater than 130 mg/dL.[13]

Inflammatory Markers: The levels of C-reactive protein (CRP) and total gamma globulin of all patients were determined by nephelometry and protein electrophoresis, respectively. Erythrocyte sedimentation rate (ESR) was determined by the Westergren method.

Statistical analysis: Descriptive analysis was used in the present study. Results are presented as mean ± SD and percentages.

RESULTS

Patients with BD (n = 38) had a mean age of 42 ± 9 years, 68% were females, and 68% Caucasian. Mean disease duration was 9.8 ± 7.5 years. Approximately 47% of the patients were taking prednisone, but at a low daily dose (7.6 ± 10.8 mg/day) (Table I).

Mean cholesterol levels were 198 ± 48 mg/dL, triglycerides 121 ± 61 mg/dL, HDL 52.4 ± 14.7 mg/dL, and LDL 119 ± 35 mg/dL. Moderate/high cardiovascular risk, according to NCEP/ATPIII criteria, were observed in 26% of the patients (n = 14) due to high cholesterol, low HDL-c in 15% (n = 5) of the patients, high LDL-c in 25% (n = 16) of the patients, and high triglyceride levels in 18% (n = 13) of the patients (Table 2).

ELISA did not detect anti-PLP antibodies in patients with Behçet's disease.

Patients with BD showed altered inflammatory markers, with mean levels of CRP of 5.95 ± 10.3 mcg/mL, and ESR within normal limits with mean levels of 14.5 ± 13.2 mm/1st hour. Additionally, 31% of the patients showed elevated CRP, and the ESR was also increased in 31% of the patients (Table 3).

DISCUSSION

The results of the present study demonstrate that patients with Behçet's disease do not have anti-lipoprotein lipase antibodies, although some of those individuals have at least one lipid at high risk levels for cardiovascular disease.

Only patients who fulfilled international criteria for BD[5] were included in this study. It is important that the strict inclusion and exclusion criteria used to select patients with BD provided a unique opportunity to define more accurately that the changes in lipid profile should be secondary to their disease.

Table 1. Demographic data and use of corticosteroids of 38 patients with Behçet's disease

	BEHÇET (n = 38)
Mean age (years)	42 ± 9
Females (%)	68%
Caucasian (%)	68%
Disease duration (years)	9.8 ± 7.5
Use of prednisone (%)	47%
Mean dose of prednisone (mg/d)	7.6 ± 10.8

Results expressed as mean ± SD or percentage (%).

Table 2. Lipoprotein and risk levels (according to NCEP/ATPIII criteria) of 38 patients with Behçet's disease

	BEHÇET (n = 38)
TC (mg/dL)	198 ± 48
HDL-c (mg/dL)	52.4 ± 14.7
LDL-c (mg/dL)	119 ± 35
TG (mg/dL)	121 ± 61
TC >200 mg/dL (%)	26.3
HDL-c <40 mg/dL (%)	15.8
LDL-c >130 mg/dL (%)	23.6
TG >150 mg/dL (%)	18.4

Results expressed as Mean ± SD or percentage TC = total cholesterol;
HDL-c = high density lipoprotein cholesterol;
LDL-c = low density lipoprotein cholesterol; TG = triglycerides.

Table 3. Inflammatory markers in 38 patients with Behçet's disease

	BEHÇET (n = 38)
CRP (mcg/mL)	5.95 ± 10.3
ESR (mm/1st hour)	14.5 ± 13.2
CRP >5 mcg/mL (%)	31.6
ESR >25 mm/1st hour (%)	31.5

Results expressed as mean ±SD or percentage (%).
CRP = C-reactive protein; ESR = erythrocyte sedimentation rate.

Therefore, drugs and comorbidities, such as diabetes, obesity, thyroid disorders, menopause, renal failure, and nephrotic syndrome represented exclusion criteria, since those factors are well known to interfere with lipid metabolism.[6-8]

Analysis of the lipid profile showed that, in patients with Behçet's disease, lipid levels represented high risk for cardiovascular diseases due to elevated levels of total cholesterol, LDL-c, and triglycerides, and low HDL-c levels. Those findings were similar to those observed in chronic inflammatory diseases associated with atherosclerosis,[14,15] such as systemic lupus erythematosus. In this context, lipoprotein lipase (LPL), the target-antigen for the antibody studied here, is known to facilitate the metabolism of plasma lipoproteins and it is the main enzyme responsible for the hydrolysis of circulating triglycerides.[1] Its hindered enzymatic activity is directly involved with the development of hypertriglyceridemia, which is known to have a role in the atherosclerotic process.[1]

Anti-LPL auto-antibodies have been implicated in the mechanisms of atherosclerosis in SLE and in other auto-immune inflammatory diseases, such as rheumatoid arthritis and systemic sclerosis.[2-4] Those antibodies have been strictly associated with the increase in triglyceride levels in SLE and scleroderma, and a putative anti-LPL functional role in those diseases derived from the observation that the anti-LPL IgG fraction of scleroderma patients with elevated serum triglyceride levels was capable of inhibiting significantly enzymatic activity *in* vitro.[4] Besides, prior studies have demonstrated that patients with active lupus show a strong correlation between circulating anti-LPL and inflammatory markers, especially CRP.[2]

Despite the presence of elevated levels of inflammatory markers, as well as altered lipid profile, in BD patients, the absence of reactivity to LPL suggests that anti-LPL antibodies are not implicated in the active, permanent, complex vascular inflammatory process that causes damages in those patients. In this aspect, it is reasonable to speculate that the inflammatory processes and dyslipidemia seen in BD and systemic connective tissue disorders (SLE and systemic sclerosis) can reflect several pathogenic mechanisms.[16] Indeed, systemic vasculitis is known to be pauci-immune conditions.

The data of the present study are conclusive in showing that anti-LPL antibodies do not seem to be implicated in the pathophysiology of the atherosclerotic inflammatory process in Behçet's disease.

REFERÊNCIAS
REFERENCES

1. Korn ED. Clearing factor, a heparin-activated lipoprotein lipase. I. Isolation and characterization of the enzyme from normal rat heart. J Biol Chem 1955; 215:1-14.

2. de Carvalho JF, Borba EF, Viana VS *et al*. Antilipoprotein lipase antibodies: a new player in the complex atherosclerotic process in systemic lupus erythematosus? Arthritis Rheum 2004; 50:3610-15.

3. Reichlin M, Fesmire J, Quintero-Del-Rio AI, Wolfson-Reichlin M. Autoantibodies to lipoprotein lipase and dyslipidemia in systemic lupus erythematosus. Arthritis Rheum 2002; 46:2957-63.

4. Kodera M, Hayakawa I, Komura K *et al*. Anti-lipoprotein lipase antibody in systemic sclerosis: association with elevated serum triglyceride concentrations. J Rheumatol 2005; 32:629-36.

5. Criteria for diagnosis of Behçet's disease. International Study Group for Behçet's Disease. Lancet 1990; 335:1078-80.

6. Attman PO, Alaupovic P. Lipid and apolipoprotein profiles of uremic dyslipoproteinemia – relation to renal function and dialysis. Nephron 1991; 57:401-10.

7. Brown WV. Lipoprotein disorders in diabetes mellitus. Med Clin North Am 1994; 78:143-61.

8. Thompson GR, Soutar AK, Spengel FA *et al.* Defects of receptor-mediated low density lipoprotein catabolism in homozygous familial hypercholesterolemia and hypothyroidism in vivo. Proc Natl Acad Sci USA 1981; 78:2591–5.

9. Siedel J, Hägele EO, Zielgenhorn J, Wahlefeld AW. Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. Clin Chem 1983; 29:1075-80.

10. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982; 28:2077-80.

11. Warnick GR, Cheung NC, Albers JJ. Comparison of current methods for high density lipoprotein cholesterol quantification. Clin Chem 1979; 25:596-604.

12. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18:499-502.

13. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001; 285:2486-96.

14. Zinger H, Sherer Y, Shoenfeld Y. Atherosclerosis in Autoimmune Rheumatic Diseases-Mechanisms and Clinical Findings. Clin Rev Allergy Immunol 2008; 8[Epub ahead of print].

15. Salmon JE, Roman MJ. Subclinical atherosclerosis in rheumatoid arthritis and systemic lupus erythematosus. Am J Med 2008; 121:S3-8.

16. Kao AH, Sabatine JM, Manzi S. Update on vascular disease in systemic lupus erythematosus. Curr Opin Rheumatol 2003; 15:519-27