European Journal of Ophthalmology / Vol. 18 no. 6, 2008 / pp. 929-933

Serum prolactin levels in HLA-B27-associated uveitis

H. PROENÇA, C. FERREIRA, M. MIRANDA, A. CASTANHEIRA-DINIS, M. MONTEIRO-GRILLO

Department of Ophthalmology, Visual Sciences Research Centre, University of Lisbon, Lisbon - Portugal

PURPOSE. *To evaluate basal serum prolactin levels in patients with HLA-B27-associated uveitis.*
METHODS. *Prospective, nonrandomized comparative trial. Thirty-three patients with HLA-B27-associated uveitis and 30 age- and sex-matched healthy control subjects were included. Age, systemic disease, treatment, and uveitis activity were recorded for comparative analysis between groups. Fourteen out of 23 patients with arthritic disease had ankylosing spondylitis. Basal serum prolactin levels were determined by electrochemiluminescence immunoassay on a Modular Analytics E170 analyzer.*
RESULTS. *Prolactinemia was significantly higher (mean=15.84 ng/mL) in patients vs controls (mean=11.50 ng/mL) (p=0.026). Subgroup analysis revealed prolactinemia in arthritic disease patients (mean=17.21 ng/mL) significantly higher than controls (mean=11.50 ng/mL) (p=0.009) and in ankylosing spondylitis (mean=17.65 ng/mL) vs controls (mean=11.50 ng/mL) (p=0.006). No correlation was found between prolactinemia and systemic treatment. Prolactinemia did not correlate with disease activity. Autoimmunity features also correlated with higher prolactinemia (mean=17.26 ng/mL) vs controls (mean=11.50 ng/mL) (p=0.015).*
CONCLUSIONS. *These results suggest the role of serum prolactin levels in HLA-B27-associated uveitis pathogenesis and its subgroups. There was no correlation with disease activity. (Eur J Ophthalmol 2008; 18: 929-33)*

KEY WORDS. *Prolactin, HLA-B27-associated uveitis, Cytokines, Arthritic disorders, Antinuclear antibodies*

Accepted: April 10, 2008

INTRODUCTION

Prolactin's (PRL) physiopathologic role knowledge has shown a remarkable development concerning immunology.
Immune function depends on the biological activities of numerous messengers termed cytokines. Cytokines play a role not only in host defense, but also in a variety of normal physiologic and metabolic processes, across a broad range of tissues.
There is conclusive scientific evidence of prolactin's functions as a cytokine (1-5). Prolactin is thymogenic and thereby influences immune cells' proliferation and differentiation. Prolactin-specific receptors have been identified on B and T lymphocytes, monocytes, and natural killer cells. There is a structural homology between receptors for PRL and for interleukins 2 and 6. Lymphocytes have also been shown to secrete a prolactin-like substance. It has been proven that hypophysectomized rats are immunocompromised and PRL introduction can restore their immune function (3). Cyclosporin A competes directly for prolactin binding sites on human lymphocytes. It also stimulates prolactin production. Both these mechanisms are responsible for cyclosporin A induced hyperprolactinemia and immunosuppression.
Dopaminergic agonists that suppress serum prolactin have been enrolled on clinical trials for autoimmune disease treatment and organ transplant rejection prevention (2, 3).
Abnormal serum prolactin levels are related to many immunologic diseases: systemic lupus erythematosus, au-

Presented at the American Academy of Ophthalmology annual meeting; Chicago; October 2005

1120-6721/929-05$25.00/0

toimmune uveitis, thyroid disease, Reiter syndrome, rheumatoid arthritis, psoriatic arthritis, juvenile chronic arthritis, Sjögren syndrome, scleroderma, dermatomyositis, multiple sclerosis, and Behçet syndrome, among others (1-6).

Prolactin shows biphasic nature of immunomodulatory effect: hypoprolactinemia and hyperprolactinemia can both lead to immunocompromise (3).

The relation of HLA-B27 and spondyloarthropathies is one of the strongest associations known between HLA and illness (7). HLA-B27-associated anterior uveitis is the most commonly diagnosed cause of anterior uveitis and noninfectious uveitis in general, accounting for 18–32% of all anterior uveitis cases in western countries (7-9). The presence of HLA-B27 has also been shown to be a predictor of anterior uveitis severity (10).

Antinuclear antibodies (ANAs) are autoantibodies directed against nuclear specificities, such as deoxyribonucleic acid or small nuclear ribonucleoproteins. ANAs serve as helpful diagnostic markers in a number of autoimmune diseases, although it lacks specificity. The indirect immunofluorescence ANAs test provides a highly sensitive screening technique for ANAs detection and is the method of choice for screening of autoimmune disease.

This study was designed to address whether patients with HLA-B27-associated anterior uveitis, or its subgroups, had abnormal basal prolactinemia. Such understanding is essential with the advent of cytokine-targeting therapies in the clinic (11).

METHODS

In this prospective, nonrandomized, comparative single-center trial, 33 patients with HLA-B27-associated uveitis followed as outpatients in our uveitis department were enrolled.

Patients were classified in subgroups according to systemic disease: patients with HLA-B27-associated arthritic disease vs patients with HLA-B27-associated nonarthritic disease.

Arthritic disorders found in the patient group included ankylosing spondylitis, overlap connective tissue disease, juvenile chronic arthritis, psoriatic arthritis, rheumatoid arthritis, and relapsing polychondritis.

Topical and systemic treatments as well as ocular disease activity were recorded for further analysis.

Patients presenting with positive antinuclear antibodies test (titer ≥1:160 by indirect immunofluorescence technique) were recorded for comparative analysis with the control group.

A group of 30 age- and sex-matched healthy subjects was used as control.

Exclusion criteria for both the patient and control groups included pregnancy, nursing, endocrinologic disease such as neoplasia (namely breast cancer or pituitary tumors), hypothalamic and pituitary stalk disease, primary hypothyroidism, chronic renal failure, cirrhosis, immunologic disease, seizures, or medications or drugs (estrogens, neuroleptics [especially phenothiazines], cimetidine, antidepressants, sulpiride, verapamil, metoclopramide, opiates, amphetamines, cocaine).

Serum prolactin levels were determined by electrochemiluminescence immunoassay on a Modular Analytics E170 Elecsys (Roche, Indianapolis, IN) analyzer.

Elecsys Prolactin used two monoclonal antibodies specifically directed against human prolactin. The test used the sandwich principle and had a total duration of 18 minutes. Results were determined via a calibration curve, which is instrument-specifically generated by two-point calibration, and a master curve, provided via the reagent barcode.

Blood sampling was performed between 8:00 and 10:00 AM, after fasting. Blood was collected using 20-Gauge needles and 5 mL syringe to standard sampling tubes and kept at ambient temperature (20–25 °C) until measurement, performed within 2 hours.

The protocol used for this study was institutionally approved.

Statistical analysis

For statistical analysis, Statistical Package for the Social Sciences (SPSS) 15.0 for Windows software was used. A descriptive analysis of demographic and clinical parameters was performed. Comparisons between groups and variables were performed using parametric Student *t*-test for independent samples. Statistical significance was assumed at $p<0.05$.

RESULTS

Thirty-three patients (mean age 45.82 years, SD=16.03, range 10–82 years, 51.50% female) with HLA-B27-associated uveitis were enrolled in this

study and underwent the baseline examination.

Subgroup classification according to systemic disease showed 23 (69.70%) patients with HLA-B27-associated arthritic disease vs 10 (30.30%) patients with HLA-B27-associated nonarthritic disease (Tab. I).

Patients with arthritic disorders included 14 (62%) with ankylosing spondylitis, 5 (22%) with overlap connective tissue disease, 1 (4%) with juvenile chronic arthritis, 1 (4%) with psoriatic arthritis, 1 (4%) with rheumatoid arthritis, and 1 (4%) with relapsing polychondritis (Tab. II).

Eleven (33%) patients were on topical ocular treatment (mainly corticosteroids). Six (18%) patients were on systemic therapy for their ocular or rheumatic disease (corticosteroids, methotrexate, sulfasalazine, or cyclosporine), 2 (6%) of which were on cyclosporine.

Eleven (33%) patients presented active uveitis at the time of PRL measurement.

Antinuclear antibodies testing was positive in 24 patients (titer ≥1:160).

In the control group, 30 subjects were recruited, mean age 44.03 years, SD=17.32, range 15–71 years, 53.30% females.

Prolactinemia was significantly higher (mean=15.84 ng/mL, SD=9.75) in patients with HLA-B27-associated anterior uveitis compared to controls (mean=11.50 ng/mL, SD=3.80) (p=0.026). Subgroup analysis revealed prolactinemia in arthritic disease patients (mean=17.21 ng/mL, SD=10.63) significantly higher than controls (mean=11.50 ng/mL, SD=3.80) (p=0.021). Patients with ankylosing spondylitis also presented with higher PRL levels (mean=17.22 ng/mL, SD=10.15) than controls (mean=11.50 ng/mL, SD=3.80) (p=0.006) (Tab. III).

No statistically significant differences were found between serum PRL levels and the other subgroups, specifically the HLA-B27-associated nonarthritic disease (mean=12.69 ng/mL, SD=2.13 vs mean=11.50 ng/mL, SD=3.80) (p=0.491). No significant differences were found comparing prolactinemia in the minor subgroups of patients with HLA-B27-associated arthritic disease.

We found significantly lower prolactinemia in patients on topical treatment vs no topical treatment (mean=10.1 ng/mL, SD=6.2 vs mean=15.9 ng/mL, SD=8.7) (p=0.043) (Tab. IV).

There were no statistically significant differences in serum PRL levels between patients on systemic treatment vs no systemic treatment (mean=19.1 ng/mL, SD=14.6 vs mean=12.9 ng/mL, SD=6.1) (p=0.304) (Tab. IV).

No correlation was found between the activity of the

TABLE I - PATIENT GROUP CLASSIFICATION

	No. (%)
HLA-B27-associated arthritic disease	23 (69.7)
HLA-B27-associated nonarthritic disease	10 (30.3)

TABLE II - ARTHRITIC DISEASE PATIENTS SUBGROUP CLINICAL CLASSIFICATION

	No. (%)	On systemic treatment, n (%)
Ankylosing spondylitis	14 (62)	4 (12)
Overlap connective tissue disease	5 (22)	0
Juvenile chronic arthritis	1 (4)	0
Psoriatic arthritis	1 (4)	1 (3)
Rheumatoid arthritis	1 (4)	1 (3)
Relapsing polychondritis	1 (4)	0

TABLE III - SERUM PROLACTIN LEVELS IN PATIENT GROUP AND MAJOR SUBGROUPS VS CONTROL

	Mean (ng/mL)	SD	p
Patients	15.84	9.75	0.026
Control	11.50	3.80	
Arthritic disease patients subgroup	17.21	10.63	0.021
Control	11.50	3.80	
Nonarthritic disease patients subgroup	12.69	2.13	0.491
Control	11.50	3.80	
Ankylosing spondylitis patients subgroup	17.22	10.15	0.006
Control	11.50	3.80	

TABLE IV - SERUM PROLACTIN LEVELS vs TREATMENT

	Mean (ng/mL)	SD	p
Topical treatment	10.06	6.24	0.043
No topical treatment	15.95	8.69	
Systemic treatment	19.06	14.57	0.304
No systemic treatment	12.86	6.14	

TABLE V - SERUM PROLACTIN LEVELS VS DISEASE ACTIVITY

	Mean (ng/mL)	SD	p
Active uveitis	14.76	8.93	0.597
Inactive uveitis	12.21	6.91	

TABLE VI - SERUM PROLACTIN LEVELS IN PATIENTS WITH POSITIVE ANTINUCLEAR ANTIBODIES TEST vs CONTROL

	Mean (ng/mL)	SD	p
Patients	17.26	10.37	0.015
Control	11.50	3.80	

uveitis and prolactinemia (active: mean=14.8 ng/mL, SD=8.9 vs inactive: mean=12.2 ng/mL, SD=6.9) (p=0.597) (Tab. V).

The proportion of cases of active uveitis did not differ comparing the arthritic disease subgroup (35%) vs nonarthritic subgroup (30%) (p=0.789).

We found significantly higher prolactinemia in patients with positive ANAs test (mean=17.26 ng/mL, SD=10.37) vs controls (mean=11.50 ng/mL, SD=3.80) (p=0.015) (Tab. VI).

DISCUSSION

The incidence of spondyloarthropathies mirrors the prevalence of HLA-B27 seropositivity. Conversely, 85% to 95% of patients with ankylosing spondylitis are HLA-B27 positive (9-12). Ankylosing spondylitis is also the most frequent systemic disease related to anterior uveitis, seen in more than 50% of spondyloarthropathies cases diagnosed after an episode of uveitis (13-15). This fact may explain why we found 15 patients with spondyloarthropathies, namely 14 with ankylosing spondylitis and 1 with psoriatic arthritis. The association of HLA-B27 alleles with Reiter syndrome, psoriatic arthritis, spondylitis in inflammatory bowel disease, enteropathic arthritis, and HIV-associated reactive arthritis is also known (16, 17).

The mean basal prolactinemia were within normal range, considering the cutoff 20 ng/mL for men and 30 ng/mL for women. However, in spite of falling out of the hyperprolactinemia cutoff values, mean PRL levels were significantly higher in patients than controls. It is also interesting that statistical significance remains when major patient subgroups are analyzed, namely patients with arthritic disease in general and patients with ankylosing spondylitis in particular. The other subgroups of HLA-B27-associated arthritic disease did not show significant PRL level differences. Whether this is due to the small number of patients must be clarified in further studies.

Sometimes patients with a history of more severe uveitis were on maintenance topical treatment although without signs of active inflammation. This may explain why there was a statistical correlation between PRL and topical treatment but not between PRL and disease activity.

In our study, PRL measurements were not correlated to systemic treatment; however, we should keep in mind the small number of patients on this therapy. On the other hand, the group being so small and only two patients on cyclosporine demonstrates that PRL measurements in our study were not biased due to systemic treatment.

Data regarding correlation of prolactinemia vs disease activity are still contradictory for many rheumatic diseases. There are no specific data available concerning HLA-B27-associated uveitis. We found no correlation between disease activity and serum PRL levels.

In this study, patients with higher PRL levels did not present particular clinical manifestations. Genetic and psychological factors in the patient group may also contribute to different clinical disease expression and disease activity.

Concerning ANAs quantified by indirect immunofluorescence ANA test, we considered a titer of 1:160 positive since it is present in only 5% of normal persons, compared with a 1:80 titer, which is present in 10–12% of normal persons (18).

Significantly higher prolactinemia in patients with positive ANA test is another clue to support PRL's role as a cytokine enrolled in immunoregulation.

These results point to the likely association between basal serum PRL levels and HLA-B27-associated anterior uveitis. Further research would be needed to elucidate the functional interactions of PRL in HLA-B27-associated anterior uveitis, clarifying pathogenesis and generating novel therapeutic options.

The authors have no conflicts of interest, proprietary or commercial interests in the products or devices mentioned in this article, or financial support.

Reprint requests to:
Helena Sofia Ferrão Mesquita Proença, MD
Hospital Santa Maria
Av. Professor Egas Moniz
1649-035 Lisboa, Portugal
helenproenca@hotmail.com

REFERENCES

1. Jara LJ, Silveira LH, Cuellar M, Pineda C, Scopelitis E, Espinosa L. Hyperprolactinemia in Reiter's syndrome. J Rheumatol 1994; 21: 1292-7.
2. Kawai T, Katoh K, Tani K. Hyperprolactinemia preceding development of autoimmune disease. J Rheumatol 1996; 23: 1483-4.
3. Reber PM. Prolactin and immunomodulation. Am J Med 1993; 95: 637-44.
4. Toubi E, Gabriel D, Golan TD. High association between hyperprolactinemia and anticardiolipin antibodies. J Rheumatol 1997; 24: 1451.
5. Walker SE, Jacobson JD. Roles of prolactin and gonadotropin-releasing hormone in rheumatic diseases. Rheum Dis Clin North Am 2000; 26: 713-36.
6. Houman H, Ben GI, Lamloum M, et al. Prolactin levels in Behçet's disease: no correlation with disease manifestations and activity. Ann Med Intern 2001; 152: 209-11.
7. Chang JH, McCluskey PJ, Wakefield D. Acute anterior uveitis and HLA B-27. Surv Ophthalmol 2005; 50: 364-88.
8. Jamil A, Thompson L, Singh S, Ahuja R, Dray P, Becker N. Incidence and causes of uveitis in a suburban general ophthalmology clinic. Invest Ophthalmol Vis Sci 2003; 44: 772-4.
9. Suhler EB, Martin TM, Rosenbaum JT. HLA-B27-associated uveitis: overview and current perspectives. Curr Opin Ophthalmol 2003; 14: 378-83.
10. Power WJ, Rodriguez A, Pedroza-Seres M, Foster CS. Outcomes in anterior uveitis associated with the HLA-B27 haplotype. Ophthalmology 1998; 105: 1646-51.
11. Rosenbaum JT, Smith JR. Anti-TNF therapy for eye involvement in spondyloarthropathy. Clin Exp Rheumatol 2002; 20: S143-5.
12. Sieper J, Braun J, Rudwaleit M, Boonen A, Zink A. Ankylosing spondylitis: an overview. Ann Rheum Dis 2002; 61: iii8-iii18.
13. Braun J, Bollow M, Remlinger G, et al. Prevalence of spondyloarthropathies in HLA-B27 positive and negative blood donors. Arthritis Rheum 1998; 41: 58-67.
14. Monnet D, Breban M, Hudry C, Dougados M, Brezin AP. Ophthalmic findings and frequency of extraocular manifestations in patients with HLA-B27 uveitis: a study of 175 cases. Ophthalmology 2004; 111: 802-9.
15. Pato E, Banares A, Jover JA, et al. Undiagnosed spondyloarthropathy in patients presenting with anterior uveitis. J Rheumatol 2000; 27: 2198-202.
16. Eastmond CJ. Genetics and HLA antigens. Baillières Clin Rheumatol 1994; 8: 263-76.
17. Reveille J, Conant M, Duvic M. Human immunodeficiency virus-associated psoriasis, psoriatic arthritis, and Reiter's syndrome: a disease continuum? Arthritis Rheum 1990; 33: 1574-8.
18. Kavanaugh A, Tomar R, Reveille J, et al. Guidelines for clinical use of the antinuclear antibody test and tests for specific autoantibodies to nuclear antigens. American College of Pathologists. Arch Pathol Lab Med 2000; 124: 71.