

Can Pentraxin-3 be a Candidate Marker in the Follow-Up of the Patients With Behçet's Disease?

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ABSTRACT

Objectives: This study aims to assess the level of pentraxin-3 (PTX-3) as an inflammatory marker and compare it with C-reactive protein (CRP) levels in patients with Behçet's disease (BD).

Patients and methods: Forty-two patients with BD (15 males, 27 females; mean age 39.7±8.6 years; range 20 to 64 years) and 42 age- and sex-matched healthy controls (14 males, 28 females; mean age 40.8±8.2 year; range 25 to 60 years) were included in the study. Serum CRP and plasma PTX-3 levels were measured. Subgroup analyses were performed according to clinical manifestations of patients with BD.

Results: Both PTX-3 and CRP levels were significantly higher in patients with BD than controls (1.33±0.29 vs 0.85±0.12, p<0.05 for PTX-3 and 0.71±0.13 vs 0.27±0.03, p<0.001 for CRP, respectively). Area under the curve was 0.633±0.062 vs 0.729±0.05, respectively. Mean PTX-3 and CRP levels were 1.1 vs 1.5, p=0.5; 0.5 vs 0.9, p=0.5; respectively, in patients with mucocutaneous involvement alone and with other involvements, whereas they were 0.9 vs 1.6, p=0.1; 0.5 vs 0.8, p=0.3; respectively, in patients with and without peripheral arthritis, and were 1.7 vs 0.9, p=0.06; 1.0 vs 0.5, p=0.07; respectively, in patients with and without uveitis.

Conclusion: Although PTX-3 levels were higher in patients with BD than healthy controls, sensitivity and specificity of PTX-3 was not different than CRP in patients with BD.

Keywords: Behçet's disease; cytokine; inflammation.

Behçet's disease (BD) is a chronic, relapsing multisystemic disease characterized with nonspecific vasculitis that affects various sizes of vessels. The highest prevalence was reported in the Eastern Mediterranean and in Asia.^{1,2} Inherited and adaptive immunity accompanied with bacterial and viral infections, activations of neutrophils, natural killers, and cytotoxic T cells were found to be responsible in the etiology.³⁻⁵ BD's association with human leukocyte antigen-B51 (HLA-B51) gene is well known, however there is no certain laboratory parameter for the diagnosis or follow-up of BD. Pentraxins (PTXs) are acute phase proteins

that affect humoral arm of congenital immunity.⁶ They are divided into two groups as short and long PTXs.^{7,8} C-reactive protein (CRP) is the prototype of the short PTX family mainly produced in the liver in response to inflammatory signals and PTX-3 is the prototype of the long PTX family.^{9,10} While CRP is produced by hepatocytes, PTX-3 is produced by different cell types and functions locally. Different from classic PTXs, PTX-3 is secreted as a response to interleukin-1 beta and tumor necrosis factor alpha. PTX-3 plays a complex role *in vivo*, recognizing a diverse range of pathogens modulating complement activity by

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binding C1q and facilitating pathogen recognition by macrophages and dendritic cells. Because of this extrahepatic synthesis in contrast to CRP, PTX-3 levels are believed to be a more reliable indicator of disease activity produced at sites of inflammation.¹¹ Increased levels of PTX-3 have been observed in some autoimmune disorders.^{11,12} In small vessel vasculitis, PTX-3 levels correlate with clinical activity of the disease and represent a candidate marker for monitoring the disease.¹¹ Nevertheless, there is only one recent report investigating PTX-3 association in patients with BD and BD's activity. Therefore, in this study, we aimed to assess the level of PTX-3 as an inflammatory marker and compare it with CRP levels in patients with BD.

PATIENTS AND METHODS

Forty-two BD patients (15 males, 27 females; mean age 39.7 ± 8.6 years; range 20 to 64 years), who attended Rheumatology Clinic at

the Department of Internal Medicine, Medeniyet University Göztepe Training and Research Hospital between January 2011 and June 2011 and were diagnosed according to the International Study Group of BD,¹³ and 42 healthy controls (14 males, 28 females; mean age 40.8 ± 8.2 year; range 25 to 60 years) were included. Exclusion criteria were disorders and habits that may affect inflammatory state (i.e. diabetes mellitus, dyslipidemia, malignancy, other rheumatologic or inflammatory disease, alcohol consumption, smoking, drug use). The present study was approved by the Ethical Board (01.04.2011 dated, decision no: 11/C) and written informed consent was obtained from all patients. The study was conducted in accordance with the principles of the Declaration of Helsinki. Morning blood samples were taken after an overnight fasting. Blood samples were centrifuged at 1,500 g for 10 minutes at room temperature within two hours after collection. Serum CRP was measured by nephelometric method (Immagine® Immunochemistry System, Beckman Coulter, USA)

Table 1. Laboratory findings of patients with Behçet's disease

	Values	Normal range
Hemoglobin (g/dL)	10.7	11.7-15.5
Hematocrit (%)	32.4	37-44
Leukocyte count (mcrL)	9.24	3.800-11.000
Platelet (mcrL)	291.000	150.000-350.000
Erythrocyte sedimentation rate (mm/h)	9	0-20
C-reactive protein (mg/L)	0.72	0-6
Urea (g/dL)	119	13-43
Creatinine (mg/dL)	1.48	0.7-1.3
Rheumatoid factor (IU/mL)	14.0	0-18
Anti-CCP (unit/mL)	0.6	0-2.5
ANA	1:>1000 granular pattern	Negative
Anti-SSA	++ Positive	Negative
Anti-SSB	+++ Positive	Negative
Ro-52 (52 Kda)	+++ Positive	Negative
ANCA	Negative	Negative
Anticardiolipin IgM/IgG	Negative	Negative
Venereal disease research-RPR test	Negative	Negative
Complement component 4 (C4) (mg/dL)	22.9	15-50
Double-stranded DNA (dsDNA) antibody	Negative	Negative
Direct Coombs test (IgG)	++++ Positive	Negative

Anti-CCP: Anti-cyclic citrullinated peptide; ANA: Antinuclear antibody; Anti-SSA: Anti Sjogren syndrome A; Anti-SSB: Anti Sjogren syndrome B; ANCA: Anti-neutrophil cytoplasmic antibody; RPR: Rapid plasma regain; dsDNA: Double stranded Deoxyribonucleic acid; IgG: Immunoglobulin G.

Table 2. Comparison of inflammatory markers between two study groups

	Healthy controls (n=42)			Patients with BD (42)			p
	n	Mean±SD	Mean±SEM	n	Mean±SD	Mean±SEM	
Gender							
Males	14			15			
Females	28			27			
C-reactive protein (mg/dL)			0.27±0.03			0.71±0.13	<0.001
Erythrocyte sedimentation rate (mm/hr)		20.1±11.0			24.3±16.5		>0.05
Pentraxin-3 (ng/mL)			0.85±0.12			1.33±0.29	<0.05

SD: Standard deviation; SEM: Standard error of mean.

as a part of daily clinical practice. Plasma (ethylenediaminetetraacetic acid-anticoagulated) samples were stored at -200 °C until analysis. Plasma PTX-3 concentration was measured by using the commercially available enzyme-linked immunosorbent assay kit (Quantikine Human Pentraxin 3/TSG-14 Immunoassay, R&D Systems, Minneapolis, USA). The analytic sensitivity of the test was 0.025 ng/mL, and the intra-assay variation coefficient (CV %) for the three separate concentrations were 3.8 (mean value: 2.61±0.01, n=20), 3.7 (mean value: 7.72±0.28, n=20), and 4.4 (mean value: 14.1±0.62, n=20)

Statistical analysis

The data were expressed as mean ± standard deviation (SD) or mean ± standard error of the mean (SEM). Statistical analysis was performed using SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Comparison between the groups were made with unpaired t-test and Mann-Whitney U test, as appropriate. Correlations were performed by the Spearman rank test. The clinical performance of CRP and PTX-3 were assessed using receiver operating characteristics curves and calculated likelihood ratios for two cut-points with either high sensitivity or high specificity. A *p* value of <0.05 was considered to be statistically significant.

RESULTS

All the patients with BD were under treatment and their clinical data can be seen in Table 1. Both CRP and PTX-3 levels were significantly higher among patients with BD than healthy controls (0.71±0.13 vs 0.27±0.03, *p*<0.001 for CRP and 1.33±0.29 vs 0.85±0.12, *p*<0.05 for PTX-3;

respectively) (Table 2). Nevertheless, increases in PTX-3 and CRP levels were similar (area under the curve: 0.633±0.062 vs 0.729±0.05; respectively) (Figure 1). Subgroup analyses were performed according to clinical features of BD. Mean PTX-3 and CRP levels were 1.1 vs 1.5, *p*=0.5; 0.5 vs 0.9, *p*=0.5; respectively, in patients with mucocutaneous involvement alone and with other involvements; whereas they were 0.9 vs 1.6, *p*=0.1; 0.5 vs 0.8, *p*=0.3; respectively, in patients with and without peripheral arthritis; and 1.7 vs 0.9, *p*=0.06; 1.0 vs 0.5, *p*=0.07; respectively, in patients with and without uveitis (Table 3).

DISCUSSION

In the present study, we determined that PTXs, both CRP and PTX-3, which are inflammation

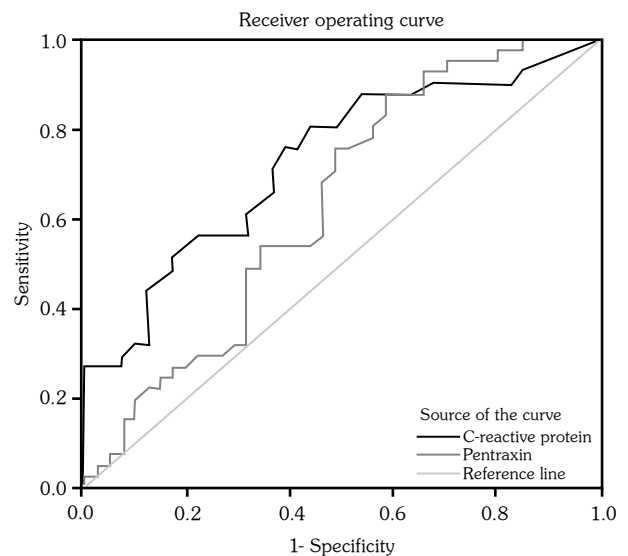


Figure 1. Sensitivities of C-reactive protein and Pentraxin-3.

Table 3. Comparison of pentraxin-3, C-reactive protein and erythrocyte sedimentation rate levels with regards to sites of involvement in patient group

	Pentraxin-3	p	CRP	p	ESR	p
With only mucocutaneous (n=18) vs with other involvement (n=26)	1 vs 1.5	0.5	0.5 vs 0.9	0.59	20 vs 27	0.5
With uveitis (n=18) vs without uveitis (n=21)	1.7 vs 0.9	0.06	1.0 vs 0.5	0.07	29 vs 19	0.07
With peripheral arthritis (n=15) vs without peripheral arthritis (n=22)	0.9 vs 1.6	0.1	0.5 vs 0.8	0.3	24 vs 25	0.3

CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate.

markers, were significantly higher in patients with BD. Nevertheless, specificity and sensitivity of PTX-3, which is a novel long PTX, were similar to those of CRP. In previous studies, it was hypothesized that PTX-3, unlike CRP, may be a rapid marker for primary local activation of innate immunity and inflammation and an indicator of disease activity while in other studies, correlation between levels of PTX-3 and CRP was found to be weak.¹⁴⁻¹⁷ In this study, PTX-3, similar to CRP, was found to be associated with BD but did not show any superiority when compared to CRP. There are limited number of studies investigating PTX-3 levels in rheumatologic diseases. In a study investigating whether PTX-3 is an indicator of small vessel vasculitis activity¹¹ in patients with Churg-Strauss syndrome, Wegener's granulomatosis, and microscopic polyangiitis, systemic lupus erythematosus, rheumatoid arthritis, and CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia), PTX-3 levels were higher in patients with untreated vasculitis and lower in patients who underwent immunosuppressive treatments ($p < 0.005$). Different from our study, PTX-3 levels did not correlate with CRP levels in patients with vasculitis.¹¹

In another study conducted on patients with rheumatoid arthritis, it was shown that PTX-3, unlike CRP, contributed to the complement-mediated mechanism causing inflammation and tissue damage. Increased levels of PTX-3 protein was obtained in synovial fluids from patients with rheumatoid arthritis. In contrast to other acute phase reactants, the majority of PTX-3 synthesis is extrahepatic and in that study the main source of PTX-3 was the synovial pannus.¹²

A study in patients with psoriatic arthritis showed a positive correlation between PTX-3 and disease activity of psoriasis. A strong PTX-3 staining in fibroblasts, endothelial cells and monocytes/macrophages was detected in severe psoriatic skin lesions.¹⁸

Recently, in a Turkish cohort of BD patients similar to our study group,¹⁹ it was determined that PTX-3 levels did not correlate with CRP or with any domains of BD's current activity form in contrast to our present study. In our study, PTX-3 levels were found to be significantly higher in patients with BD. Since most of the patients of our cohort were in remission, we tried to find out the difference of PTX-3 levels according to BD's site-specific involvement, rather than disease activity. PTX-3 levels did not show any difference according to involvement. We only observed a slight increase in patients who had uveitis. However, this increase was not statistically significant. Our study indicates that PTX-3 can be an alternative parameter to CRP in the follow-up of BD. However, we need further studies to determine the relationship between PTX-3 and severity of BD.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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