Serum eosinophil cationic protein levels in Behçet's disease and its relation to clinical activity

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Summary

Background: **Eosinophil cationic protein (ECP) is a matrix protein of eosinophils and has been reported to reflect eosinophil activity. Few studies have examined the role of eosinophils in the pathogenesis of Behçet's disease.**

Objective: **The purpose of the present study was to investigate the serum ECP levels in BD and its relation to clinical activity.**

Methods: **Forty-seven consecutive patients with BD (22 active, 25 inactive), 21 age and sex matched patients with allergic rhinitis and 21 healthy controls were evaluated cross-sectionally. The serum ECP levels were measured by the flourescein enzyme immunoassay method.**

Results: **Mean serum ECP levels of active patients with BD (34.28 ± 23.43 μg/L) were found to be significantly lower than those of the inactive patients (65.69 ± 46.32 μg/L, p <0.05) and the controls (62.92 ± 30.49 μg/L, p <0.01) . Behçet patients with oral aphthous lesions had significantly lower mean serum ECP levels (n=21, 38.82 ± 33.38 μg/L) than those without aphthous lesions (n=26, 60.81 ± 43.21μg/L) (p = 0.041). Similarly patients with arthritis had lower serum ECP values (n=6, 22.12 ± 9.47 μg/L) than those without arthritis (n = 41, 55.21 ± 41.35 μg/L) (p =0.029).**

Conclusions: **Lower ECP levels in the active phase of the disease may be a result of decreased production due to the activation of Th1 cytokines.** ***(Asian Pac J Allergy Immunol 2012;31:67-72)***

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Introduction

Behçet's disease (BD)[1] is a systemic vasculitis, characterized by recurrent oral aphthae, genital ulcers, uveitis, skin lesions, arthritis, gastrointestinal and neurologic manifestations. It has a substantial morbidity primarily from eye and neurologic involvement and may be fatal due to vascular complications.[2]

There has been a growing interest in research on the pathogenesis of the disease. Recent studies have revealed the central role of T cell-mediated immune responses in the pathogenesis of BD.[3,4] Increased Th1 activity has been shown in patients with active BD.[3] Several phenotypical and functional abnormalities were reported in both αβ+ and γδ+ T cells.[4] Neutrophils were also implicated in the pathogenesis of BD.[5] Hyperactive neutrophils have been suggested to play an important role in the pathogenesis of the vascular lesions.[6] Some investigators have reported increased number of NK cells in patients with clinically active Behçet's disease.[7] Altered innate immune function, such as mannose binding lectin deficiencies or alterations of toll-like receptor expression, has also been reported.[8-10]

Eosinophils have been implicated in the vascular injury associated with several vasculitis syndromes, such as Churg Strauss syndrome,[11] Temporal arteritis[12] and Henoch-Schonlein purpura.[13] In Behçet patients, several conflicting results are reported in the literature. Dinc et al. have found that serum IgE levels and eosinophils counts of Behçet patients are comparable with those of controls.[14] Chang et al. have shown that the prevalence of atopy and atopic diseases is significantly lower in BD patients than in controls. Other atopy parameters, such as serum IgE levels and peripheral blood eosinophil counts, were also significantly lower in BD patients when compared with controls. Atopy, serum IgE levels, and peripheral blood eosinophil counts did not differ significantly

between BD patients with and without severe manifestations. They concluded that these data support the hypothesis that "Th-1 cell-mediated disorders protect against the development of Th-2 cell-mediated diseases".[15] In a study conducted by Korkmaz, it was reported that the total number of eosinophils in the peripheral blood and peripheral blood film was not different in Behçet patients and controls. However, serum IgE was higher in Behçet patients. The author commented that this could be attributible to the thrombosis in Behçet disease, as the IgE could take part in platelet activation via mast cells or basophils.[16] Onat et al. have reported that Behçet's patients commonly exhibit elevated plasma IgE levels. However, elevated IgE levels are not correlated with levels of acute phase reactants or disease activity and they suggest that IgE levels might be an independent diagnostic clue in Behçet's patients.[17] Scully et al have studied serum immunoglobulin levels in Behçet disease. Only IgA concentrations were significantly higher but the rest (IgG, IgM, IgD or IgE) were comparable with the controls.[18]

Eosinophil cationic protein (ECP) is a matrix protein of specific granules of eosinophils with considerable capacity to damage tissue and cells[19] and has been reported to reflect eosinophil activity.[19,20] Increased serum levels of ECP has been reported in patients with seasonal allergic rhinitis.[21] Several immunomodulatory features of ECP have been reported, such as inhibition of the proliferative T-lymphocyte response to antigens,[19] immunoglobulin production, proliferation of plasma cell lines[22] and upregulation of ICAM-1.[19] Procoagulant effects of ECP have also been reported.[12,23]

In a study reported by our group, lower serum ECP levels were found in patients with BD when compared to controls but this difference was attributed to the usage of concomitant medications such as colchicine and in some patients to corticosteroids and/or cyclosporine. It was also reported that serum ECP levels were increased in the active state of disease compared to the inactive phase.[26]

Serum ECP concentrations correlate very well with blood eosinophil counts. ECP has been studied in a number of inflammatory diseases, as mentioned above. It has also been studied in Behçet disease, but only during drug treatment. The present study was conducted in patients not taking confounding drugs that can effect serum ECP levels. The purpose of this study was to investigate the serum ECP levels in BD and its relation to the clinical activity.

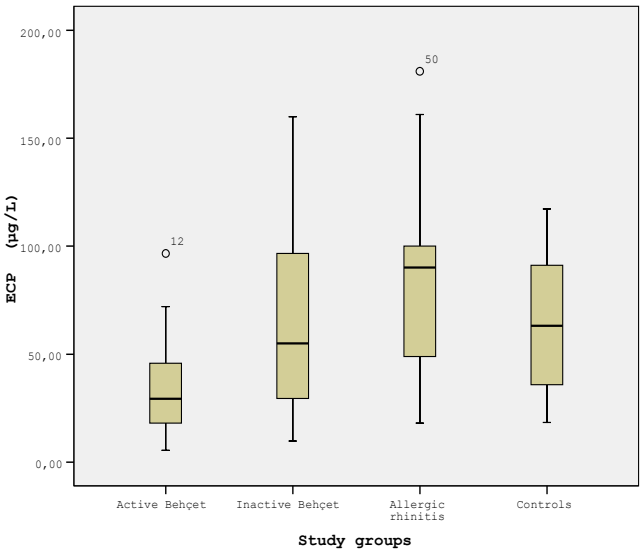

Figure 1. Serum ECP (eosinophil cationic protein) levels for the study groups

Methods

Forty-seven consecutive patients with BD (22 active, 25 inactive) meeting International Study Group Criteria,[24] 21 age and sex matched patients with allergic rhinitis and 21 apparently healthy controls were evaluated prospectively in the Rheumatology-Immunology Outpatient Clinic. Clinical activity was defined according to the criteria proposed by "Behcet's Disease Research Committee of Japan".[4]

Patients on corticosteroids or immunosuppressives were excluded. Colchicine was stopped 10 days prior to the blood collection in inactive patients. Blood was drawn from the active patients before starting treatment. Cases with any allergy history or parasitosis were excluded.

The study was approved by the Ethics Committee. Written informed consent was taken from all of the participants and study was performed in accordance with the principles of the Declaration of Helsinki.

Venous blood was drawn from antecubital fossa between 10-12 a.m. into serum seperation tubes with clot activator and gel barrier as recommended in the literature.[25] After the blood samples had been kept at room temperature for an hour, they were centerfuged at 3000 rpm for 4 minutes. Serum

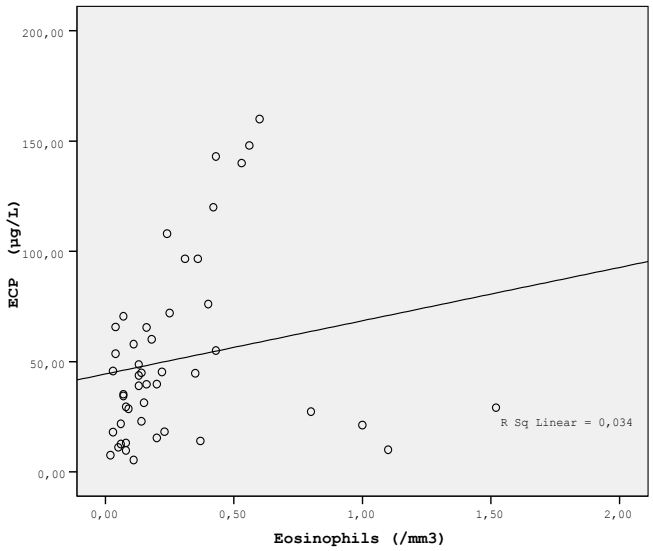

Figure 2. The correlation between ECP (eosinophil cationic protein) and eosinophil counts in Behçet patients

samples were transfered into Eppendorf tubes and refrigerated at -20° C until the assay time.

Tubes were defrosted at room temperature on the assay day. Serum ECP measurement was done using Unicap ECP flourescein enzyme immunoassay (FEIA) kits (Pharmacia & Upjohn Diagnostics AB Uppsala, Sweden) by CAP System (Pharmacia Unicap 100) with a reference interval of 2-200 µg/L. Total IgE was measured by "Dade Behring 100 Analyzer" nephelometer using "Dade Behring" kits, with reference values between 0.0-180.0 IU/ml.

Absolute eosinophil counts was assesed in the complete blood count measured by a Beckman Coulter Gen S System II. Erythrocyte sedimentation rate was measured by the Westergren method. C-reactive protein (CRP) was measured by highly sensitive CRP kits using the "Dade Behring 100 Analyzer", having 0.0-6.0 mg/L of normal reference values.

For statistical evaluation, nonparametric tests were used. Mann Whitney-U, Chi-square test, Kruskal Wallis and Spearman correlation tests were performed by using SPSS 19 statistical software program.

Results

The demographic and laboratory data for the study groups are shown in Table 1. Serum ECP levels of active patients with BD were found to be significantly lower than those of the inactive patients and the controls. Behçet patients also had significantly lower ECP and IgE values when compared to the allergic rhinitis group (Table 3). Organ involvements of the patients are summarized in Table 2.

Table 1. Demographic and laboratory characteristics of the study groups

	Behcet Disease med (min-max) mean ± sd	Active Behcet Group med (min-max) mean ± sd	Inactive Behcet Group med (min-max) mean ± sd	Allergic Rhinitis med (min-max) mean ± sd	Controls med (min-max) mean ± sd
Age (years)	38 (16-66) 37.1±9.4	35 (16-66) 34.9±11.6	41 (26-52) 39.0±6.5	35 (17-58) 34.2±10.0	34 (26-45) 34.0±5.5
Gender (Number) (female/male)	24 / 23	12/10	12/13	12/9	11/10
Disease duration (years)	8 (1-25) 8.6 ± 5.9	6 (1-22) 7.3 ± 5.5	8 (1-25) 9.8 ± 6.2	NA	
Family history	Positive in 9 patients	Positive in 4 patients	Positive in 5 patients	NA	
CRP (mg/L)	4.2 (0.0-90.5) 11.6 ± 16.0	7.2 (3.5-90.5) 14.6 ± 20.4	3.9 (0.0-45.2) 9.0 ± 10.3	3.5 (3-6.4) 4.13 ± 1.08	6 (2.2-83.7) 14.8±20.1
ESR (mm/h)	14 (2-91) 20.4 ± 18.0	16.5 (5-91) 22.7 ± 21.2	14 (2-67) 18.5 ± 14.8	15 (1-37) 15 ±10	9 (1-34) 11±8
HLA B5	10/22 (46.8 %)	2 / 7 (28.5 %)	8/15 (53.3 %)	NA	NA

Table 2. Clinical manifestations of the Behçet patients*

	Active patients	Inactive patients	*p*-value
Oral aphthae	19	3	0,000
Genital ulcers	3	1	0,2
Eye involvement	9	2	0,01
Skin involvement	15	5	0,000
Deep venous insufficiency	6	8	0,4
Arthritis	6	0	0,008
Neurologic involvement	1	0	0,4
CRP (mg/L) mean(min-max)	7,1 (3,5-90,5)	3,9 (0,0-45,2)	0,2

* The organ systems not included in the table had no involvement

Behçet patients with oral aphthae at presentation had significantly lower mean serum ECP levels (n=21, 38.82 ± 33.38 μg/L) than those without aphthous lesions (n=26, 60.81 ± 43.21μg/L) (p = 0.041). Similarly Behçet patients with arthritis had lower serum ECP values (n=6, 22.12 ± 9.47 μg/L) than those without arthritis (n=41, 55.21 ± 41.35 μg/L) (p =0.029). Serum total IgE levels were also lower in Behçet patients with arthritis when compared to patients without arthritis (52.33 ± 84.60 IU/ml (n =6) vs 165.78 ±21.98 IU/ml (n =41))(p = 0.029).

Serum ECP levels showed weak to moderate correlations with IgE levels and absolute eosinophil counts in Behçet patients (Table 4). ECP levels did not show any correlation with the clinical activity scores, CRP or ESR.

Discussion

A number of studies pertaining to the pathogenesis of Behçet's disease have been conducted, however little has been done concerning eosinophil behaviour in this vasculitis. This study was designed to show the eosinophil activity by measuring serum ECP levels in Behçet's disease.

The mean serum ECP levels in patients with active BD were found to be significantly lower than those of inactive patients, as well as the control group. Lower serum ECP levels were also associated with some clinical findings, namely oral aphthous lesions and arthritis.

This study is important because the subjects were selected without the interference of any confounding medication, in contrast to a former study.[26]

Table 3. Mean serum eosinophil cationic protein (ECP), immunoglobulin E (IgE) levels and absolute eosinophil counts for the study groups

	ECP (μg/L)	Eosinophils (/mm^3)	IgE (IU/ml)
Behçet Disease (n=47)	50.99±40.26‡	152.6±130.4	151.3 ±194.2‡
Active (n=22)	34.28±23.43†,*	116.8±96.3	130.8±175.0
Inactive (n= 25)	65.69±46.32	184.1±149.2	169.4±211.6
Allergic rhinitis (n= 21)	65.69±46.32	184.1±149.2	169.4±211.6
Controls (n=21)	62.92±30.49	183.8±145.7	53.8±33.1

*: p <0.05, compared to inactive Behçet patients

†: p <0.01, compared to controls

‡ : p <0.01, compared to the allergic rhinitis group

A vast amount of research have been conducted on the serum ECP levels and its relation to the clinical activity in several rheumatologic conditions. Konca et al. have reported increases of ECP in FMF (Familial Mediterranean patients), when compared to an asthmatic control group and they have attributed this increase to the predominant role of the Th2 subset of lymphocytes.[27] Five fold increases in serum ECP levels in patients with rheumatoid arthritis compared to healthy controls has been reported and the highest ECP levels have been measured in patients with an aggressive course.[28] Four fold increases in serum ECP levels have been reported in patients with systemic sclerosis, compared to healthy controls, and impairment of pulmonary function tests were correlated with

Table 4. Correlations of serum eosinophil cationic protein (ECP) levels with C-reactive protein (CRP), absolute eosinophil counts and immunoglobulin E (IgE) levels

	Correlations	r
Behcet patients (n=47)	ECP-eozinofil	0.357*
	ECP-IgE	0.428†
Active (n=22)	IgE-CRP	0.427*
Inactive (n=25)	ECP-IgE	0.554†
	ECP-eozinofil	0.621‡

*: p <0.05, †: p <0.01, ‡: p ≤0.001

bronchoalveolar lavage ECP levels.[29] Activated eosinophils and secreted ECP have been seen in all layers of the inflammed vessels and cytotoxic eosinophil granules have been suggested to contribute to necrotic lesions and the development of thrombi in patients with temporal arteritis.[12]

In the present study, ECP levels in active patients with BD were found to be significantly lower than those of the inactive patients and healthy controls, whereas eosinophil counts were comparable. In these groups, the findings may imply that eosinophil function may change, despite stable eosiophil counts in BD.

There are several possible explanations for the lower ECP levels in BD patients in this study. They may be attributed to decreased production, which may be the result of the Th1 activation in active Behçet patients.[23] A second explanation may be the adherence of positively charged ECP to the negatively charged membrane fragments in injured vessels in the active stage of the disease. A further study of tissue ECP may reveal the possible bound fraction of ECP to the injured vessels.

Measurement of ECP is easy and inexpensive and can be performed in routine laboratories.

This study suffers from a number of limitations; the principal of them is relatively small number of individuals available for analysis. Serum IL-5 measurements would give more clue as to the effect of the cytokine network on serum ECP levels. As ECP has been implicated in the pathogenesis of thrombus in temporal arteritis studied in biopsy specimens,[12] examination of tissue ECP from thrombotic lesions of BD may be an interesting topic for further studies. A dynamic observation of ECP level along with the clinical organ involvements in BD would more clearly define the relationships. Despite its limitations this study may offer an insight into the role of eosinophils in the inflammatory process of BD.

In summary serum ECP levels seem to be negatively associated with active disease. However there are several conflicting results in the literature. Therefore, confirmation of ECP as a negative response marker of activation needs further studies.

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