

Level of Plasma Elastase - $\alpha 1$ - Proteinase Inhibitor in Patients with Behçet's Disease

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Background : The common histopathology of Behçet's disease is vasculitis associated with activation of neutrophils. The level of plasma elastase - $\alpha 1$ - proteinase inhibitor (E - $\alpha 1$ - PI), which represents the activation of neutrophils, may be a marker of Behçet's disease.

Objective : We examined the level of plasma elastase - $\alpha 1$ - proteinase inhibitor to evaluate the degree of neutrophil activation in Behçet's disease.

Method : We measured plasma elastase - $\alpha 1$ - proteinase inhibitor in 34 cases of untreated Behçet's disease patients and 30 cases of normal individuals by an enzyme immunoassay. We also studied the differences between the levels in two clinical types of Behçet's disease, the complete and incomplete type.

Results : The plasma level of elastase - $\alpha 1$ - proteinase inhibitor was significantly higher in untreated Behçet's disease patients than in healthy controls. However, there was no significant difference between the levels in the two clinical types.

Conclusion : These data suggest that the elevated level of plasma elastase - $\alpha 1$ - proteinase inhibitor may reflect a state of chronic activation of neutrophils in Behçet's disease immunologically and further studies will be needed to evaluate the clinical status of Behçet's disease patients by measuring levels of plasma elastase - $\alpha 1$ - proteinase inhibitor.

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Key Words : Behçet's disease, Elastase - $\alpha 1$ - proteinase inhibitor, Neutrophil

INTRODUCTION

The common histopathology of Behçet's disease is vasculitis, and the pathogenesis of the vessel damage in Behçet's disease remains controversial. Many authors have assessed the neutrophil function in patients with Behçet's disease by using various methods, and demonstrated circulating immune complexes and a factor in serum that enhances

polymorphonuclear leukocyte (PMNL) migration¹, enhanced chemotactic activity of PMNL², and increased chemotactic activity and minor alterations in functional metabolic activity of PMNL³. Neutrophil elastase is a kind of neutrophil proteinase, present in the azurophilic granules of the neutrophils, which has a broad enzymatic activity and is originally involved in the digestion or lysis of bacteria and foreign bodies, but causes tissue injury when in excess. So, it is considered to be involved in tissue injury in a variety of inflammatory diseases⁴. Released neutrophil elastase is unstable and immediately forms a complex with $\alpha 1$ - proteinase inhibitor ($\alpha 1$ - PI = $\alpha 1$ - antitrypsin)^{4,5}. Therefore the level of plasma elastase - $\alpha 1$ - proteinase inhibitor (E - $\alpha 1$ - PI) can reflect the level of elastase liberated from the activated neutrophils

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and can be a good indicator for the systemic and localized activation of the granulocyte⁶⁻¹¹. We examined the levels of plasma E- α 1-PI to evaluate the degree of activation of neutrophils in the pathogenesis of Behçet's disease, and compared the levels between the two clinical types of Behçet's disease, the complete and incomplete type.

MATERIALS AND METHODS

We studied 34 patients with Behçet's disease. The patients had not received any treatment during the previous 3 months. There were 22 males and 12 females. In the control group, there were 30 healthy subjects (20 males and 10 females). The mean age of the patients was 30.7 years and that of control group was 34.2 years. The diagnosis of Behçet's disease was made according to the criteria of the International Study Group for Behçet's disease¹² and the patients with Behçet's disease were classified by Shimizu's classification¹³. Based on this classification, there were 13 cases of the complete type and 21 cases of the incomplete type. Venous blood was obtained from all subjects and the plasma was stored at -70°C until analyzed. The levels of plasma E- α 1-PI were measured by enzyme immunoassay (PMN-Elastase assay, Merck, Darmstadt, Germany). The levels of E- α 1-PI in the plasma were determined by the method of Neumann et al, which is a sandwich method of using antibodies against elastase as the primary antibody to coat the tubing wall and alkaline phosphatase (AP)-labeled anti- α 1-PI antibody was used as the secondary antibody. Under these conditions the used activity of AP towards p-nitrophenyl-phosphate is proportional to the concentration of E- α 1-PI in the sample¹¹. With this method, mass concentrations of E- α 1-PI were measured but neither concentration nor activity of elastase itself were determined. Statistical significance was analyzed with the Mann-Whitney test for non-parametric data and Pearson's coefficient for correlation.

RESULTS

As shown in Table 1, the level of plasma elastase- α 1-proteinase inhibitor was 49.6 ± 16.3 $\mu\text{g/l}$ in the control subjects and 743.8 ± 343.6 $\mu\text{g/l}$ in the patients with untreated Behçet's dis-

ease. Thus, the levels of plasma elastase- α 1-proteinase inhibitor in patients with untreated Behçet's disease were significantly higher than those in the controls. There was a marked difference statistically between the levels of plasma elastase- α 1-proteinase inhibitor in patients with untreated Behçet's disease and the control group ($p < 0.01$). For each clinical type, the level of plasma elastase- α 1-proteinase inhibitor was 745.5 ± 346.8 $\mu\text{g/l}$ in the complete type and 742.8 ± 350.2 $\mu\text{g/l}$ in the incomplete type. There was no significant difference between the levels in the two clinical complete and incomplete types.

DISCUSSION

Behçet's disease is a complex multisystem disease and the etiology remains enigmatic. A genetic predisposition is supported by the strong association with HLA-B51¹⁴. No primary infectious etiology has been supported, however, some studies suggest patients have heightened immune responses to antigenic components of certain microorganisms such as streptococci, *Mycobacterium tuberculosis*, herpes simplex virus, Epstein-Barr virus, and human immunodeficiency virus. Underlying abnormalities in T-lymphocyte function may be integral to this aberrant response¹⁵. Recently many authors describe that cellular and humoral immunological mechanisms seem to be involved cardinally in the pathogenesis of Behçet's disease¹⁶⁻¹⁹. The common histopathology of Behçet's disease is vasculitis, and some investigators have demonstrated the presence of immune complex mediated neutrophilic vasculitis (leukocytoclastic vasculitis) in Behçet's disease^{1,20,21}, others have demonstrated lymphocytic vasculitis in cutaneous lesions^{20,21}, mononuclear cell infiltration without vessel wall immunoglobulin deposition in the early stage of pathergic lesions^{19,23}. As previously mentioned, studies also have been concerned with a role for circulating immune complexes and neutrophils in the pathogenesis of mucocutaneous lesions in Behçet's disease^{1-3,18,20}.

Neutrophil elastase, present in the azurophilic granules of the neutrophils, is a physiologically important enzyme originally involved in the digestion or lysis of bacteria and foreign bodies. However, when the excessive release of this enzyme from the cells occurs, it acts with other neutral proteases

Table 1. Level of plasma elastase - $\alpha 1$ - proteinase inhibitor ($\mu\text{g/l}$)

Subjects	E- $\alpha 1$ -PI ($\mu\text{g/l}$)
Controls (n=30)	49.6 \pm 16.3
Behçet's disease (n=34)	743.8 \pm 343.6 *
Complete type (n=13)	745.5 \pm 346.8 *
Incomplete type (n=21)	742.8 \pm 350.2 *

Values are mean \pm S.D.

* $p < 0.01$, compared with normal controls

(collagenase, cathepsin G) and myeloperoxidase to break down plasma proteins or connective components in the tissue matrix, or produce a variety of physiologically active substances, thus exerting a toxic effect on the living body⁴. It is presumed that this enzyme is involved very importantly in the maintenance of homeostasis of the body fluid components such as the complement-immunological system and coagulation fibrinolytic system by attacking the blood complement components, immunoglobulins, and coagulation - fibrinolytic factors^{4,6,8}. Recent investigations indicated that neutrophil elastase had multifunctional activities for blood coagulation and fibrinolysis. It inactivates coagulation factors II, V, VII, VIII, XI, XII, and XIII and $\alpha 2$ - plasmin inhibitor by proteolytic cleavage, and exhibits direct fibrinolytic activity by digesting fibrin and fibrinogen^{4,8,10}. On the other hand, the activities of this enzyme are controlled by potent proteinase inhibitors.

When elastase is released from the activated neutrophils, $\alpha 1$ - PI, a main physiologic inhibitor of neutrophil elastase, binds and inactivates the elastase very rapidly. Therefore, activity of elastase is normally restricted to the microenvironment of the activated neutrophils⁴. The $\alpha 1$ - PI is known to act as an inhibitor of serine proteinases with activity against a large range of enzymes such as pancreatic and neutrophil elastase, pancreatic trypsin and chymotrypsin, skin and synovial collagenases, elastin, thrombin, kallikrein, and acrosin²⁴. Ninety % of the activities of neutrophil elastase are inhibited by $\alpha 1$ - PI and the remaining 10% are inhibited by $\alpha 2$ - macroglobulin^{5,9}. Because $\alpha 1$ - PI and $\alpha 2$ - macroglobulin are present in excess, direct measurement of the neutrophil elastase activities in plasma is difficult. However, increased levels of E - $\alpha 1$ - PI would be an indication of elastase liber-

ation^{6,7,10}. The plasma levels of E - $\alpha 1$ - PI have been reported to be increased in other diseases, such as rheumatoid arthritis⁶, myelocytic leukemia⁷, leprosy⁸, and septicemia⁹. Kiyohiro et al suggested that plasma E - $\alpha 1$ - PI levels appeared to be a useful marker of thromboembolic vasculopathy in Behçet's disease¹⁰. We showed that the mean plasma level of E - $\alpha 1$ - PI, which represents the activity of neutrophils, is significantly increased in patients with untreated Behçet's disease compared to that of control subjects. Therefore the elevated levels of plasma E - $\alpha 1$ - PI may reflect a state of chronic activation of neutrophils in Behçet's disease immunologically and be a useful marker in Behçet's disease. However, there were no significant differences between the levels of plasma E - $\alpha 1$ - PI in complete and incomplete types of Behçet's disease. The reason for this may be that the sample size was not large enough or the diagnostic criteria by Shimizu et al. serve better for purposes of classification than discrimination²⁵. However, we could not compare the levels of plasma E - $\alpha 1$ - PI among all four clinical types by Shimizu's classification. We think further studies are necessary to evaluate the mechanism of neutrophil activation in Behçet's disease and to compare the levels of plasma E - $\alpha 1$ - PI of Behçet's disease patients in relation to the clinical activity, acute or remission phase, and thrombosis, that is, the acute phase reactant levels, hemostatic variables, and others should be measured.

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