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Serum levels of IgG antibodies against alpha-enolase are increased in patients with Behçet's disease and are associated with the severity of oral ulcers

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Abstract

Serum levels of IgG antibodies against alpha-enolase are increased in patients with Behçet's disease and are associated with the severity of oral ulcers

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Behcet's disease (BD) is a chronic inflammatory disease of unknown etiology, characterized by recurrent oral and genital ulcers, skin lesions, uveitis, and arthritis. It is regarded as vasculitis and anti-endothelial cell antibodies (AECA) are found in patients with BD. One of the endothelial cell antibodies was reported to recognize alpha-enolase. This study aimed to investigate the association between anti-alpha-enolase antibody (AEA) and clinical manifestations or disease activity of BD. To measure serum AEA level in patients with BD, we performed Western blot analysis and ELISA assays. AEA was more frequently found in patients with BD (82.4%) than in healthy controls (66.7%) on Western blots. Serum AEA titers were

increased in BD patients compared to healthy controls (1578.2 ± 115.7 vs. 1149.9 ± 134.3 AU, p = 0.037) and were correlated with the number of oral ulcers in BD patients (Spearman's coefficient = 0.275, p = 0.012). BD patients with oral ulcers were divided into mild (0~2 oral ulcers) and severe (greater than 2 oral ulcers) groups based on the cumulative number of oral ulcers in the 4 weeks prior to blood sampling. Patients in the severe group exhibited significantly higher AEA titer than did those with mild oral ulcers or healthy controls (Kruskal-Wallis test; p = 0.001). Skin lesions, uveitis, and arthritis were not associated with serum AEA levels. Cell surface alpha-enolase expression was examined using flow cytometry to investigate the correlation with serum AEA titer. Frequency of surface α -enolase-expressing cells was increased in patients with BD in several cell types, including lymphocytes and monocytes. Alpha-enolase expression in lymphocytes was correlated with serum AEA titer (Spearman's correlation coefficient = 0.417, p = 0.034). In conclusion, serum AEA titer is increased in BD patients and is associated with the number of oral ulcers. These results suggest that AEA may be useful as a serological marker of Behçet's disease, particularly in patients with severe oral ulcers.

Keywords : anti-alpha-enolase antibody (AEA), alpha-enolase (α-enolase), Behçet's disease (BD), oral ulcer.

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Abbreviations

AEA anti-alpha-enolase antibody

AECA anti-endothelial cell antibody

APC allophycocyanin

BD Behçet's disease

BDCAF Behçet's Disease Current Activity Form

BSA bovine serum albumin

BSAS Behçet's Syndrome Activity Score

ELISA Enzyme-linked immunosorbent assay

ESR Erythrocyte sedimentation rates

FBS fetal bovine serum

FITC Fluorescein isothiocyanate

HC healthy control

HRP horseradish peroxidase

hs-CRP high sensitivity C-reactive protein

OU oral ulcer

PBMC peripheral blood mononuclear cell

PerCP peridinin chlorophyll

PBS phosphate buffered saline

PE Phycoerythrin

PE-Cy7 Phycoerythrin-cyanin7

RA Rheumatoid arthritis

SEM standard error of the mean

SLE Systemic lupus erythematosus

SPSS Statistical Product and Service Solutions program

TMB tetramethylbenzidine

TNF-alpha (TNF-α) tumor necrosis factor alpha

Contents

Abstract ····· i

Abbreviations ····· iii

Contents ····· v

List of tables and figures ····· vii

Introduction ····· 1

Materials and methods ····· 4

Patients and controls ····· 4

Cell isolation ····· 5

Western blot analysis ····· 5

Enzyme-linked immunosorbent assay (ELISA) of anti-alpha-enolase antibody ····· 6

Flow cytometry analysis ····· 7

Statistical analysis ····· 8

Results ····· 9

Anti-alpha-enolase antibody titer in sera from patients with BD

versus healthy controls ····· 9

Correlation of serum AEA with clinical manifestations in patients with BD ····· 10

Increased expression of cell surface alpha-enolase in PBMCs from patients with BD ····· 11

Discussion ····· 24

References ····· 28

Abstract in Korean ····· 34

List of tables and figures

Table 1 Clinical and laboratory features of patients with BD and healthy controls ····· 13

Table 2 Correlation between anti-alpha-enolase antibody and clinical manifestations or disease activity ····· 14

Table 3 Correlation between the expression of alpha-enolase on the surface of lymphocytes or monocytes and clinical manifestations or disease activity in patients with BD ····· 15

Figure 1 Western blot analysis of human sera. Recombinant human alpha-enolase protein was applied to the membrane ····· 16

Figure 2 Serum anti-alpha-enolase antibody titer in BD patients and healthy controls ····· 17

Figure 3 Comparison between the number of oral ulcers and the titer of serum AEA in patients with BD ····· 18

Figure 4 Serum levels of AEA in patients with BD according to the number

of oral ulcers ····· 19

Figure 5 Expression of alpha-enolase on the surface of peripheral blood mononuclear cells (PBMCs) from healthy controls and BD patients ····· 20

Figure 6 Comparison of alpha-enolase on the surface of lymphocytes, monocytes, or neutrophils in patients with BD and healthy controls ····· 21

Figure 7 Comparison of surface expression of alpha-enolase in the subsets of lymphocytes, T cells and B cells, in BD patients and healthy controls ····· 22

Figure 8 Correlation between alpha-enolase positivity in lymphocytes and serum AEA levels ····· 23

Introduction

Behcet's disease (BD) is a chronic, multisystemic inflammatory disease with clinical manifestations that include recurrent oral and genital ulcers, skin lesions, uveitis, and arthritis (James, 1979; Al-Otaibi et al, 2005). The etiology of the disease remains to be elucidated. There are no specific laboratory findings or serological markers of BD, so diagnosis is made conducted exclusively based on clinical manifestations (1990).

Several autoantibodies have been found in patients with Behçet's disease, including antibodies against endothelial cells, Annexin V, HSP-70, and C1q (Aydintug et al, 1993; Aslan et al, 2004; Birtas-Atesoglu et al, 2008; Bassyouni et al, 2014). Among them, anti-endothelial cell antibodies (AECA) are regarded as one of the primary targets in BD (Lee et al, 2003; Kalayciyan et al, 2007). The characteristics of target proteins of AECA are obscure, because it is a heterogeneous family of antibodies that recognize different proteins on the endothelial cell (Belizna et al, 2006). Recently, human alpha-enolase (α-enolase) on the surface of endothelial cells has been reported as a target antigen of AECA by a study examining several candidate proteins in BD (Lee et al, 2003).

Alpha-enolase, a multifunctional protein, is abundant in the cytosol of most cells and is a key glycolytic enzyme. Alpha-enolase is expressed on the surface of many eukaryotic cells after exposure to certain inflammatory

stimuli through unknown mechanisms (Giallongo et al, 1986). Human haematopoietic cells, including lymphocytes, monocytes and neutrophils, express alpha-enolase on their surfaces in response to exogenous phorbol myristate acetate and lipopolysaccharides (Fontan et al, 2000). Overexpression of cell-surface alpha-enolase has been reported in several autoimmune diseases, such as rheumatoid arthritis (RA), vasculitis, systemic lupus erythematosus (SLE) and Crohn's disease (Fontan et al, 2000; Pancholi, 2001).

The precise role of alpha-enolase in the pathogenesis of BD is still not clear. However, environmental factors, such as bacterial infection, endothelial injury, and UV exposure, have been shown to induce the expression of alpha-enolase on the cell surface and lead to formation of autoantibodies in autoimmune disease states (Kalayciyan et al, 2007).

There have been several studies on anti-alpha-enolase antibodies (AEA) in systemic autoimmune and inflammatory diseases (SLE, systemic sclerosis, rheumatoid arthritis, and multiple sclerosis), and in cancer-associated retinopathy syndrome (Terrier et al, 2007; Rattner et al, 1991; Moodie et al, 1993; Roozendaal et al, 1998; Dot et al, 2005).

This study aimed to investigate whether serum IgG-type anti-alpha-enolase antibodies are correlated with clinical manifestations and disease activity of BD. In addition, the surface expression of alpha-enolase was measured in haematopoietic cells, including lymphocytes, monocytes, and neutrophils, in

order to analyze its association with serum AEA levels.

Materials and methods

Patients and controls

Peripheral blood was obtained from 83 patients with BD, who fulfilled the classification criteria of the International Study Group for BD (Tunc et al, 2001) and 34 age/sex-matched healthy controls. Clinical information on organ involvement during the 4 weeks prior to blood sampling was prospectively obtained (recurrent oral and genital ulcers, skin lesions such as erythema nodosum and folliculitis, ocular lesions, and arthralgia). Laboratory measurements including erythrocyte sedimentation rates (ESR) and high sensitivity C-reactive protein (hs-CRP) levels were also examined. Patients with BD were categorized according to the number of oral ulcers (mild group, $\leq$ 2 oral ulcers; severe group, $>$ 2 oral ulcers in the previous 4 weeks). Two disease activity questionnaires were administered, the Behçet's Disease Current Activity Form (BDCAF) (Bhakta et al, 1999) and the Behçet's Syndrome Activity Score (BSAS). The BDCAF assesses disease activity based on current symptoms and is a composite index of patient and physician assessments of disease scored on a scale of 0 to 12. The BSAS is based solely on patient reports and also assesses current symptoms; it is scored on a scale of 0 to 100. On both questionnaires, higher scores indicate more active disease state. This study was approved by the Institutional Review Board of Seoul National University Hospital.

Cell isolation

Peripheral blood mononuclear cells (PBMCs) were obtained from anticoagulant-treated blood by density gradient centrifugation using Ficoll-Paque Plus (GE Healthcare Bioscience, Uppsala, Sweden). Samples were mixed with an equal volume of phosphate buffered saline (PBS) and then carefully layered on Ficoll. After centrifugation at 890×g for 20 minutes, PBMCs were collected and washed twice with PBS. Neutrophils were taken from the bottom layer following density gradient centrifugation. After lysis of red blood cells by ammonium chloride (StemCell Technologies, Vancouver, Canada) treatment for 10 minutes on ice, the pellet was processed as described previously.

Western Blot analysis

To identify IgG-type anti-alpha-enolase antibody in the serum, 0.1 or 0.2 μg recombinant human alpha-enolase (Prospec, NessZiona, Israel) was loaded onto a 12% polyacrylamide gel (Thermo Scientific, Waltham, MA), and electrophoresis was performed. The gel was transferred to a polyvinylidene difluoride membrane. The membranes were blocked overnight at 4°C with PBS containing 0.05% Tween 20 (PBST) and 3% bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO). The sera from healthy controls and BD patients, diluted 1:100 with PBST containing 1% BSA, were treated as

primary antibody and incubated overnight at 4°C. Rabbit anti-human alpha-enolase antibody (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA) was used as a positive control. After washing with Tris-buffered saline (TBS) containing 0.1% Tween 20, membranes were incubated with horseradish peroxidase (HRP)-conjugated anti-human IgG antibody (1:5000, Merck-Millipore, Darmstadt, Germany) or HRP-conjugated anti-rabbit antibody (1:5000). Immunoreactive bands were visualized with an electrochemiluminescence (Merck-Millipore) detection system (LI-Cor Biosciences, Lincoln, NE).

Enzyme-linked immunosorbent assay (ELISA) of anti-alpha-enolase antibody

Serum anti-alpha-enolase antibody levels were measured by enzyme-linked immunosorbent assay (ELISA). Recombinant human alpha-enolase (Prospec) was coated onto microtiter plates (Corning Inc., Corning, NY) with 50 mM sodium carbonate coating buffer overnight at 4°C. The plates were washed three times with PBST and incubated with PBS containing 1% BSA to block non-specific binding for 1 hour at 37°C. Then, 100 μl of sera from healthy controls and BD patients, diluted 1:50 in PBST with 0.1% Triton X-100 and 0.5% BSA, were serially diluted (two-fold steps) and applied to different wells and the plates were incubated for 2 hours at 37°C. After

washing the plates, peroxidase-conjugated goat anti-human IgG antibody (Merck-Millipore), diluted 1:5000 in PBST containing 0.5% BSA, was added to each well and the plates were incubated for 1 hour at 37°C. Antibody binding was detected by the addition of tetramethylbenzidine (TMB) with H_2O_2 (BD Biosciences) as a substrate. The color reaction was stopped with 2*N* H_2SO_4, and the plates were read spectrophotometrically at 450 nm on an ELISA reader (Bio Tek, Winooski, VT). Data were measured by titer, defined as the inverse value of the greatest dilution that still showed a positive result.

Flow cytometry analysis

Isolated PBMCs were resuspended in staining buffer containing 0.5% fetal bovine serum (FBS) (BD Biosciences), and non-specific binding was blocked by treatment with human Fc blocking reagent (BD Biosciences) for 10 minutes at room temperature. Then, the samples were stained for 20 minutes on ice in the dark with the following fluorescent-conjugated antibodies : anti-CD3 peridinin chlorophyll (PerCP) (mouse IgG1,κ, clone SK7), anti-CD11b/Mac-1 phycoerythrin-cyanin7 (PE-Cy7) (mouse IgG1,κ, clone ICRF44), anti-CD14 PE (mouse IgG2b,κ, clone MφP9), anti-CD16 allophycocyanin (APC) (IgG1,κ, clone B73.1), anti-CD20 PE (mouse IgG1,κ, clone L27) (all from BD Biosciences), and anti-alpha-enolase fluorescein

isothiocyanate (FITC) (goat polyclonal) (Santa Cruz Biotechnology). Cells were washed twice with staining buffer and analyzed using a LSRFortessa instrument and FACSDiva software (all from BD Biosciences). 200,000 events were collected for each analysis. Flow cytometric data were analyzed with FlowJo software, version 10.0.7 (Tree star inc., Ashland, OR). Lymphocytes were gated by size and complexity and monocytes were gated by CD14 positivity from PBMCs. Neutrophils were gated by CD16 and CD11b double-positivity in granulocytes extracted by different gradients from PBMCs.

Statistical analysis

Data from individual experiments are expressed as the means ± standard error of the mean (SEM). Nonparametric Mann-Whitney U tests and Kruskal-Wallis tests were performed to determine significant differences between groups, and Fisher's exact test was used for categorical variables. Correlations were examined by Spearman's correlation coefficient. *P* values less than 0.05 were considered statistically significant. All statistical analyses were performed using SPSS, version 21 (SPSS Inc., Chicago, IL). Graphs were created using Origin software, version 9.1 (OriginLab Corp., Northampton, MA).

Results

Anti-alpha-enolase antibody titer in sera from patients with BD versus healthy controls

The clinical and laboratory findings in patients with BD are shown in Table 1. To identify IgG-type AEA, Western blotting was performed. Recombinant human alpha-enolase was applied to the membrane, which was then incubated with sera from BD patients or healthy controls and treated with HRP-conjugated anti-human IgG antibody. AEA was present in 14 (82.4%) of 17 BD patients as compared to 6 (66.7%) of 9 healthy controls (Figure 1). Quantitative analysis of serum AEA was done by ELISA. BD patients showed higher AEA titers than did healthy controls (1578.2 ± 115.7 vs. 1149.9 ± 134.3 AU, $p = 0.037$) (Figure 2).

Correlation of serum AEA with clinical manifestations in patients with BD

We analyzed the correlation between clinical manifestations, laboratory tests, and disease activity in BD patients with serum AEA levels (Table 2). The number of oral ulcers was significantly correlated with serum AEA titer (Spearman's coefficient = 0.275, p = 0.012) (Figure 3). Other clinical manifestations and disease activities, such as BDCAF and BSAS scores, were not correlated with serum AEA levels. We divided the patients with BD into two groups according to the number of oral ulcers experienced in the previous 4 weeks, severe (> 2 ulcers) or mild ($\leq$ 2 ulcers). BD patients in the severe group had higher AEA titers than did those in the mild group (p = 0.002) and the healthy controls (p = 0.001) (Kruskal-Wallis test; p = 0.001) (Figure 4).

Increased expression of cell surface alpha-enolase in PBMCs from patients with BD

We examined alpha-enolase expression in the cell membrane using flow cytometry. We compared the levels of surface alpha-enolase on PBMCs from healthy controls (n = 12) to those from patients with BD (n = 17) (Figure 5). PBMCs from BD patients had more alpha-enolase on the cell surface than did those from healthy controls. Next, we examined surface alpha-enolase levels in several types of haematopoietic cells (lymphocytes, monocytes, and neutrophils) from the peripheral blood of BD patients and healthy controls (Figure 6). On the surface of lymphocytes and monocytes, the expression levels of alpha-enolase were significantly elevated in patients with BD (Mann-Whitney U test; p = 0.018 and p = 0.038, respectively). Lymphocytes were subdivided into T cells and B cells for analysis, and we found no significant difference in the expression of alpha-enolase between the subsets (Figure 7). Then, the percentage of alpha-enolase positive lymphocytes was compared with serum AEA titer (BD patients, n = 17; healthy controls, n = 9). Alpha-enolase expression on the surface of lymphocytes was correlated with serum AEA titer (Spearman's correlation coefficient = 0.417, p = 0.034 (Figure 8). However, the number of alpha-enolase-positive monocytes was not correlated with serum antibody titer (Spearman's correlation coefficient = 0.192, p = 0.346). The correlation

between alpha-enolase positive lymphocytes or monocytes and the clinical manifestations or disease activity in BD patients was examined (Table 3). However, there was no correlation between alpha-enolase positivity in PBMCs and clinical manifestations or disease activity.

Table 1 Clinical and laboratory features of patients with BD and healthy controls

	BD patients (n = 83)	Healthy controls (n = 34)
Age, years	47.6 ± 1.2	43.9 ± 2.3
Sex, female (%)	51 (61.4)	24 (70.6)
Disease duration, years	8.9 ± 0.9	N/A
Symptom frequency		
Oral ulcer	85.5	N/A
Genital ulcer	16.9	N/A
Erythema nodosum	44.6	N/A
Folliculitis	41.0	N/A
Arthralgia	51.8	N/A
Uveitis	8.3	N/A
History of uveitis	32.1	N/A
History of enteritis	10.7	N/A
ESR (mm/h)	22.8 ± 2.0	N/A
hs-CRP (mg/dL)	0.4 ± 0.1	N/A
BDCAF	6.4 ± 0.3	N/A
BSAS	25.7 ± 1.5	N/A
Medications		
Colchicine	60 (71.4)	N/A
Corticosteroid	32 (38.1)	N/A
Prednisolone equivalent dose (mg/day)	3.2 ± 6.1	N/A
Immunosuppressant	31 (36.9)	N/A

Data are presented as mean (± SEM) for continuous variables and number (percentage) for categorical variables.

BD = Behçet's disease; BDCAF = Behçet's Disease Current Activity Form; BSAS = Behçet's Symdrome Activity Score; ESR = erythrocyte sedimentation rate; hs-CRP = high sensitivity C-reactive protein; Immunosuppressant: sulfasalazine, cyclosporine, tacrolimus, azathioprine, or mycophenolate mofetil.

Table 2 Correlation between anti-alpha-enolase antibody and clinical manifestations or disease activity

Parameter	Correlation coefficient	P value
Age	0.187	0.091
Oral ulcers	**0.275**	**0.012***
Genital ulcers	-0.036	0.749
Erythema nodosum	-0.021	0.850
Folliculitis	-0.031	0.779
ESR	0.183	0.111
hs-CRP	0.055	0.642
BDCAF	-0.025	0.824
BSAS	0.100	0.372

Spearman's rho was used; *$p < 0.05$

ESR = erythrocyte sedimentation rate; hs-CRP = high sensitivity C-reactive protein; BDCAF = Behçet's Disease Current Activity Form; BSAS = Behçcet's Syndrome Activity Score.

Table 3 Correlation between the expression of alpha-enolase on the surface of lymphocytes or monocytes and clinical manifestations or disease activity in patients with BD

Parameter	Lymphocytes		Monocytes	
	Correlation coefficient	P value	Correlation coefficient	P value
Oral ulcers	0.209	0.437	-0.109	0.687
Genital ulcers	-0.149	0.582	-0.474	0.064
Erythema nodosum	0.365	0.164	0.324	0.221
Folliculitis	0.243	0.365	-0.169	0.532
ESR	0.052	0.850	-0.032	0.905
hs-CRP	0.133	0.637	0.131	0.642
BDCAF	0.164	0.545	-0.213	0.428
BSAS	0.125	0.644	-0.094	0.729

Spearman's rho was used.

ESR = erythrocyte sedimentation rate; hs-CRP = high sensitivity C-reactive protein; BDCAF = Behçet's Disease Current Activity Form; BSAS = Behçet's Syndrome Activity Score.

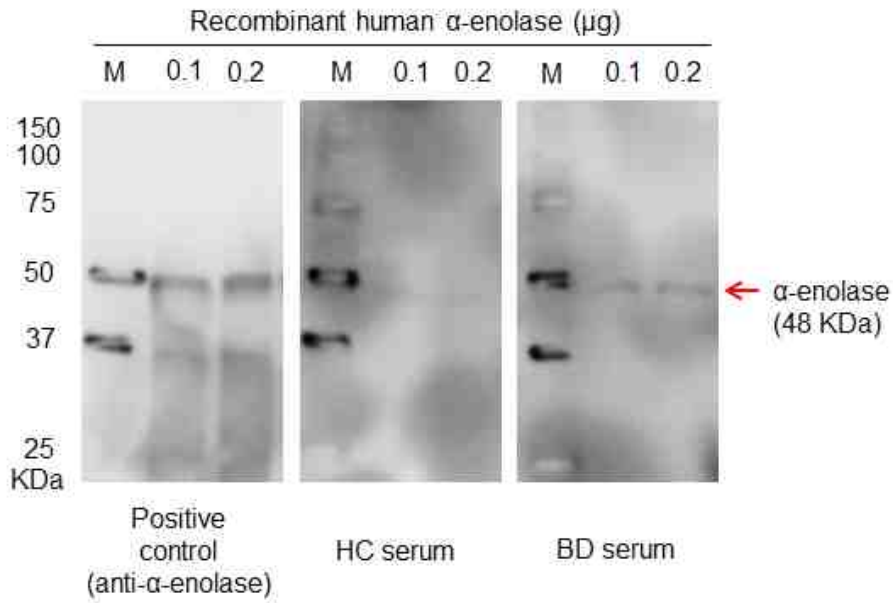

Figure 1 Western blot analysis of human sera. Recombinant human alpha-enolase (α-enolase) protein was applied to the membrane. Positive control data was obtained using rabbit anti-human alpha-enolase antibody. In contrast to healthy control sera, a specific band at 48 KDa corresponding to the recombinant human alpha-enolase protein, was detected in the positive controls and sera from BD patients. This is representative data.

BD = Behçet's disease; HC = healthy control; M = protein size marker.

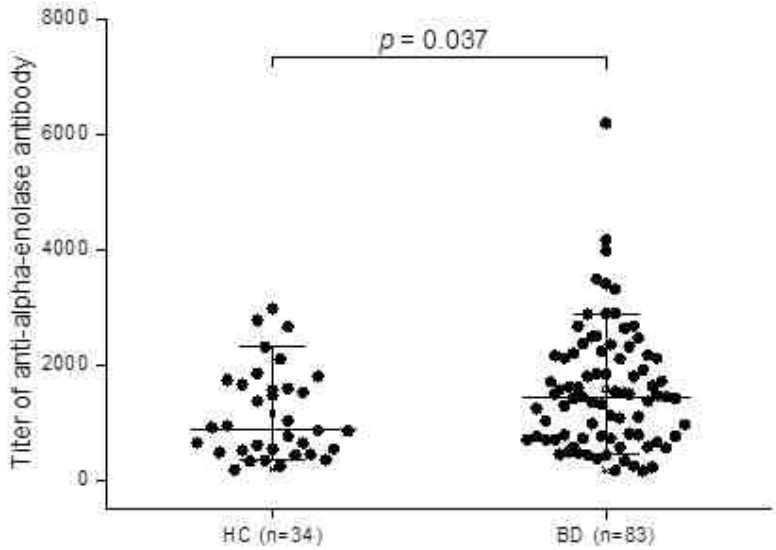

Figure 2 Serum anti-alpha-enolase antibody titer in BD patients and healthy controls. Serum AEA levels were significantly increased in BD patients compared with healthy controls ($p = 0.037$). The lines in the middle indicate the median; the bars indicate the 10th and 90th percentiles.
Mann-Whitney U test was used; $*p < 0.05$
BD = Behçet's disease.

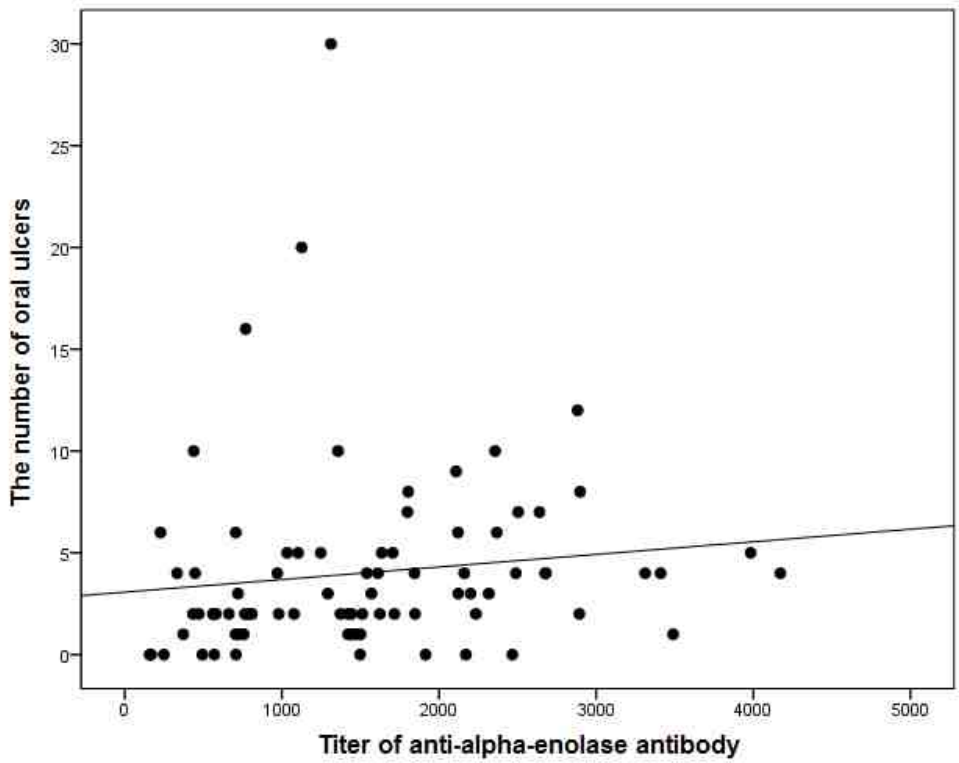

Figure 3 Comparison between the number of oral ulcers and the titer of serum AEA in patients with BD. Serum AEA titer was positively correlated with the number of oral ulcers (Spearman's coefficient = 0.275, p = 0.012).

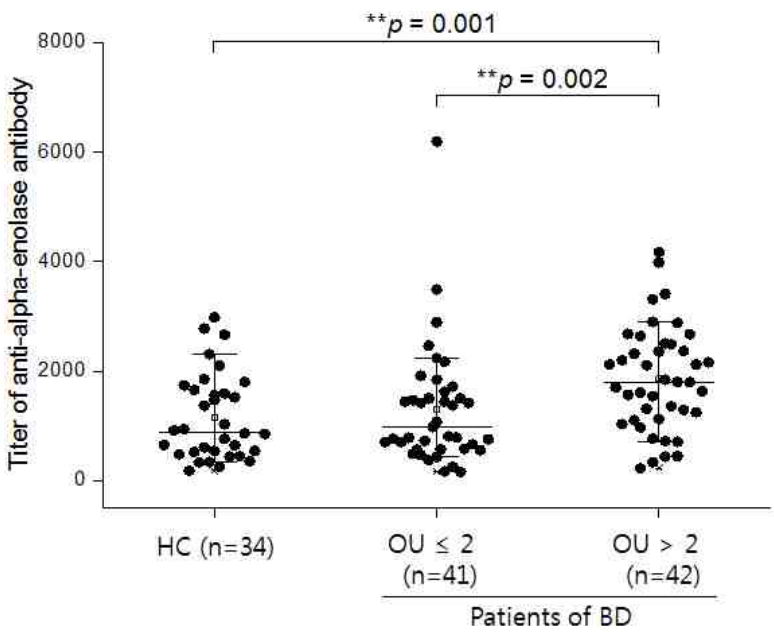

Figure 4 Serum levels of AEA in patients with BD according to the number of oral ulcers. Significantly increased serum AEA levels were found in the severe group (> 2 oral ulcers) compared with the mild group (≤ 2 oral ulcers) (p = 0.002) or healthy controls (p = 0.001). The lines in the middle indicate the median; the bars indicate the 10th and 90th percentiles (Kruskal-Wallis test; p = 0.001).

Mann-Whitney U test was used; *p < 0.05; **p < 0.01

HC = healthy controls; OU = oral ulcer; BD = Behçet's disease.

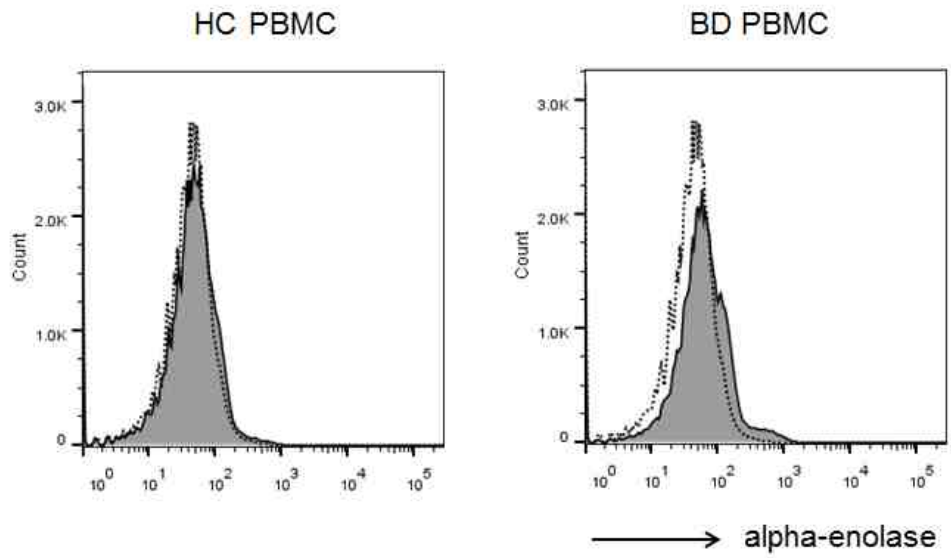

Figure 5 Expression of alpha-enolase on the surface of peripheral blood mononuclear cells (PBMCs) from healthy controls and BD patients. Expression of alpha-enolase on the surface of PBMCs from patients with BD was significantly greater than in healthy controls. Open histogram indicates isotype control (negative control); closed histogram indicates the positively stained cells.

HC = healthy controls; BD = Behçet's disease.

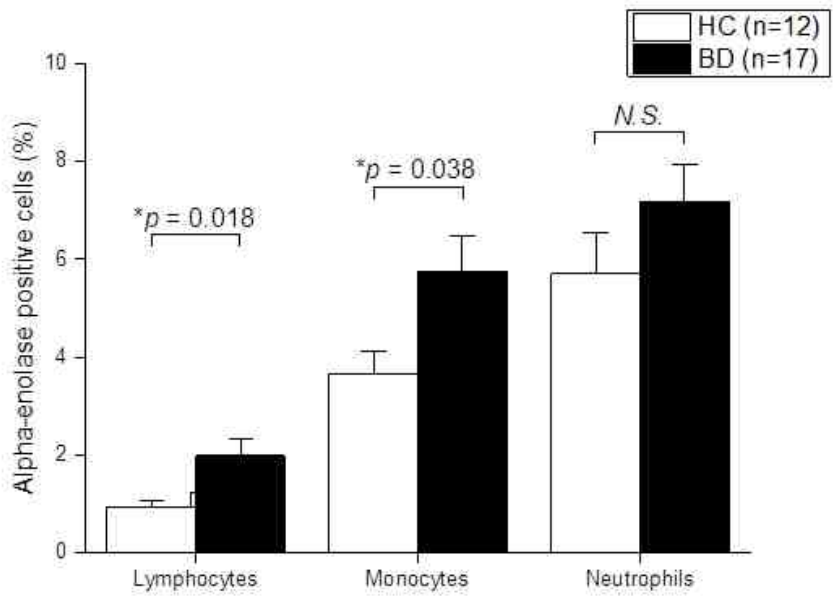

Figure 6 Comparison of alpha-enolase on the surface of lymphocytes, monocytes, or neutrophils in patients with BD and healthy controls. The expression of alpha-enolase was increased in lymphocytes and monocytes from BD patients compared to healthy controls. Values are presented as mean ± SEM.

Mann-Whitney U test was used; $*p < 0.05$

HC = healthy controls; BD = Behçet's disease; *N.S.* = no significance.

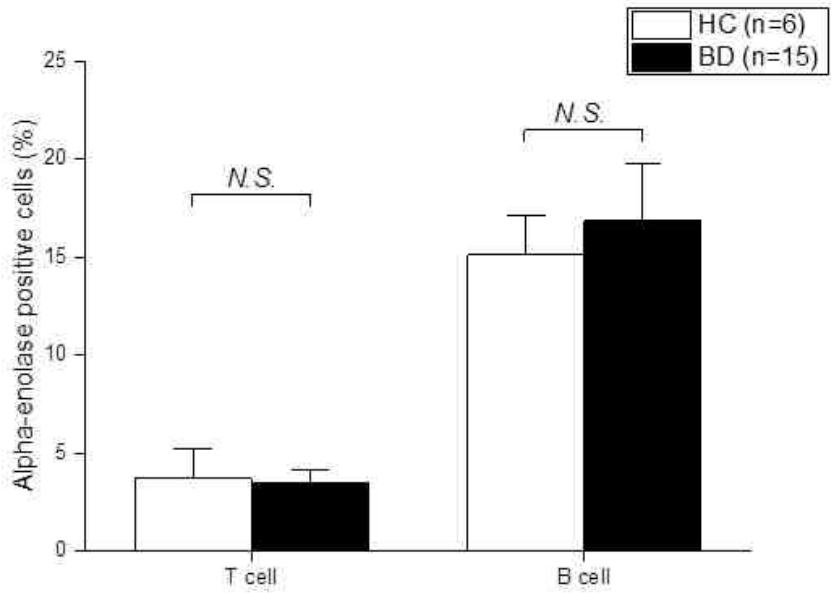

Figure 7 Comparison of surface expression of alpha-enolase in the subsets of lymphocytes, T cells and B cells, in BD patients and healthy controls. There was no significant difference between the patients with BD and healthy controls in T cell or B cell alpha-enolase expression (p = 0.235 , p = 0.910). Values are presented as mean ± SEM.

HC = healthy controls; BD = Behçet's disease; *N.S.* = no significance.

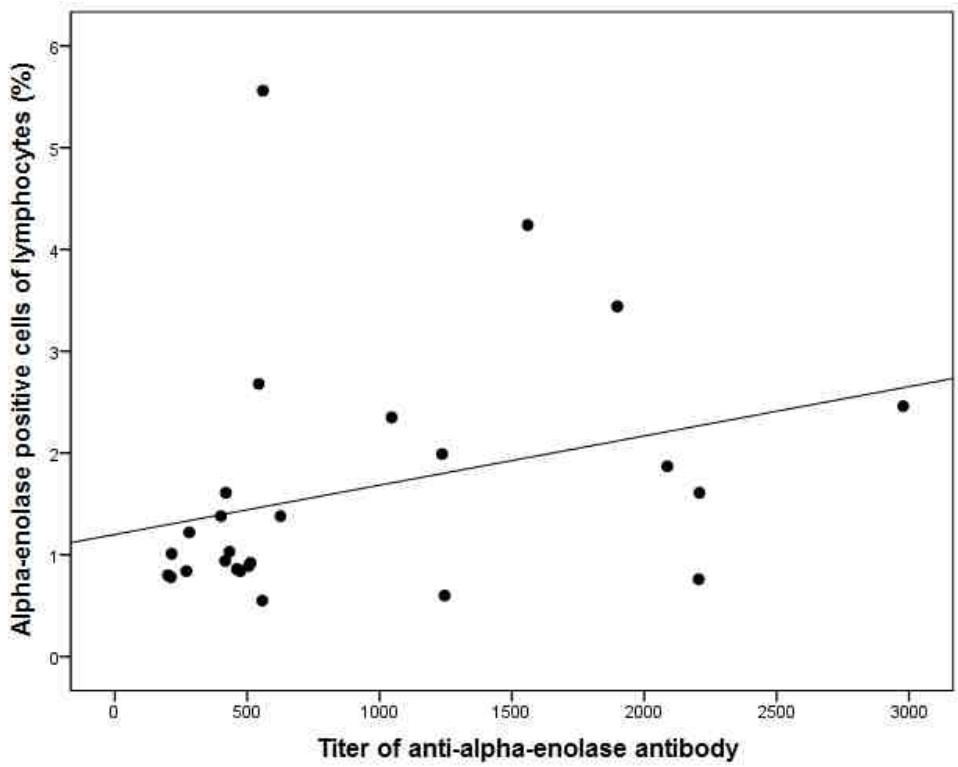

Figure 8 Correlation between alpha-enolase positivity in lymphocytes and serum AEA levels. Alpha-enolase positivity in lymphocytes was strongly correlated with serum AEA levels (Spearman's correlation coefficient = 0.417, $p = 0.034$).

Discussion

Behçet's disease (BD) is characterized by systemic vasculitis. Endothelial cells are regarded as one of the primary targets in BD (Lee et al, 2003; Kalayciyan et al, 2007). AECA have been detected in diseases associated with vascular injury, such as systemic vasculitis, rheumatoid vasculitis, and Behçet's disease (Aydìntug et al, 1993; Meroni et al, 1995). The characteristics of the antigens that bind AECA are not well understood because AECA targets heterogeneous proteins on the surface of endothelial cells (Belizna et al, 2006). Recently, alpha-enolase was reported to be one of the target antigens of AECA in BD (Lee et al, 2003). That study investigated the characteristics of alpha-enolase on the surface of endothelial cells and its serum antibody levels. The levels of serum IgM-type anti-alpha-enolase antibodies (AEA) were increased in BD patients with ocular lesions or thrombosis. In intestinal BD, a type of inflammatory bowel disease (IBD), levels of IgM anti-alpha-enolase antibodies were increased and were correlated with the scores on the Harvey-Bradshaw index (HBI), the disease severity index used in assessing Crohn’s disease (Shin et al, 2011).

We investigated the IgG-type anti-alpha-enolase antibody, which could play a role in the pathogenesis of BD. Previous reports indicated that IgG anti-alpha-enolase antibodies were detected more frequently in patients with

various systemic autoimmune disorders, such as SLE, systemic sclerosis, and RA, than in healthy controls (Pratesi et al, 2000), but its levels had not yet been measured in patients with BD.

We measured serum IgG-type AEA levels and analyzed their correlation with disease activity in BD. The titer of IgG-type AEA was increased in patients with BD compared to healthy controls. We also found a correlation between serum antibody titer and the number of oral ulcers.

IgM AEA, an AECA family member, was previously associated with the involvement of the vascular system in BD (Lee et al, 2003; Shin et al, 2011). However, our data showed that the titer of IgG AEA was not associated with the involvement of the vascular system, such as uveitis and erythema nodosum. This may imply that different isotypes of AEA have different functions depending on inflammation site.

Enolase, a multifunctional protein, is abundantly found in the cytosol of most cells. In mammals, the enzyme has 3 subunits, α, β, and γ, each encoded by a separate gene, which can combine to form homodimers or heterodimers, thereby creating different isoenzymes (Pancholi, 2001). In human cells, the three homodimeric forms are common (αα, or enolase-1, β β, or enolase-3, and γγ, or enolase-2) (Marangos et al, 1978). The isoenzymes are found in different locations and have different functions.

Alpha-enolase, which is expressed in most tissues, catalyzes the dehydration of 2-phospho-D-glycerate to phosphoenolpyruvate during the ninth step of

glycolysis. Besides its well-known glycolytic role, alpha-enolase has diverse functions and is located in many different sites, so it presumably affects various biological and pathophysiological processes (Pancholi, 2001). Tau-crystallin, a major lens protein in vertebrates, shows a similarity with the sequence of human alpha-enolase (Wistow et al, 1988). The *alpha-enolase* gene can be alternatively spliced into a nuclear form, Myc-binding protein-1, which inhibits the *c-myc* protooncogene (Subramanian et al, 2000). In addition, alpha-enolase was identified as a heat shock protein in *Saccharomyces cervesiae* (Iida et al, 1984).

Alpha-enolase can exist in the surface of a variety of cells as a plasminogen-binding receptor (Miles et al, 1991). The mechanism of translocation to the cell membrane remains unknown. Some activated immune cells and tumor cells express alpha-enolase on the cell membrane. Plasminogen binds to alpha-enolase and then plasmin, the activated form of plasminogen, enhances fibrinolysis and contributes to tissue invasion, metastasis, and so on (Liu et al, 2007).

We analyzed several types of immune cells to investigate the expression of surface alpha-enolase for the following reasons: lymphocytes are activated in patients of BD (Direskeneli, 2001); various proinflammatory cytokines are produced by monocytes such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α) in the serum of BD patients (Sahin et al, 1996); and neutrophils are hyperactive in BD (Jorizzo et al, 1995).

Alpha-enolase expression on the surface of heamatopoietic cells was increased in patients with BD compared to healthy controls, especially in lymphocytes. The levels of alpha-enolase in lymphocytes were correlated with the serum antibody titer. Although a low proportion of lymphocytes expressed alpha-enolase, the presence of alpha-enolase in the membrane may contribute to the formation of AEA.

Although the pathological role of AEA is not fully understood in BD, it may bind to several types of immune cells that express alpha-enolase on their surface and induce those cells to produce proinflammatory cytokines such as TNF-α, IL-1, and interferon-γ (IFN-γ) (Bae et al, 2012).

In conclusion, serum anti-alpha-enolase antibody levels are increased in patients with BD and that increase is correlated with the number of oral ulcers. Additionally, we found that serum antibody titer correlated with alpha-enolase expression on the cell surface of lymphocytes.

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Abstract in Korean

베체트병은 전신성 혈관염으로 분류되는 만성 염증성 질환으로, 재발성 구강궤양, 생식기 궤양, 피부 병변, 포도막염, 관절염등의 증상을 동반한다. 베체트병의 원인은 아직 불명확하나 내피세포의 성분인 알파 에놀레이즈가 자가항원으로 제시되었다. 본 연구는 베체트병 환자를 대상으로 혈청 항 알파 에놀레이즈 항체 역가를 측정하고 베체트병의 임상적 특성이나 질병 활성도와 관련이 있는지를 조사하였다. 혈청 내 항체를 측정하기 위해 Western blot과 효소면역분석법 (ELISA)을 사용하였고, 또한 말초혈액단핵세포에 존재하는 여러 면역세포의 세포표면에 항체 타깃 분자인 알파 에놀레이즈의 발현을 비교 분석하였다. 혈청 항 알파 에놀레이즈 항체는 건강 대조군에 비해 베체트병 환자에서 통계적으로 유의하게 높게 발현되었고 (1578.2 ± 115.7 vs. 1149.9 ± 134.3 AU, p = 0.037), 이는 다양한 임상적 특성 중 구강궤양의 개수와 양의 상관관계를 보였다 (Spearman's coefficient = 0.275, p = 0.012). 베체트병 환자를 구강궤양의 개수 2개를 기준으로 질병의 중증도를 나누어서 분석 했을 때, 2개 이하의 경한 그룹에 비해, 2개 초과되는 중증 그룹에서 항 알파 에놀레이즈 항체가 높게 발현되는 것을 확인하였다 (Kruskal-Wallis test; p = 0.001). 다른 임상적 특성이나 질병 활성도와 항 알파 에놀레이즈 역가의 연관성은 보이지 않았다. 또한 말초혈액에 존재하는 여러 면역세포 중, 림프구

와 단핵구의 세포표면에 알파 에놀레이즈 단백질이 베체트병 환자에서 건강 대조군에 비해 유의하게 더 많이 발현되었다. 특히 알파 에놀레이즈를 발현하는 림프구의 비율은 혈청 내 항 알파 에놀레이즈 항체 역가와 양의 상관관계를 보였다 (Spearman's correlation coefficient = 0.417, p = 0.034). 결론적으로 베체트병 환자에서 혈청 항 알파 에놀레이즈 항체는 증가되어 있고, 그 역가는 구강궤양의 중증도를 판단하는 지표로 사용 될 수 있을 것으로 생각된다.