What is the Role of Cutaneous Lymphocyte Antigen in the Pathogenesis of Behçet's Disease: A Randomized, Prospective Double-Blinded Study

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Abstract: *Background*: Behcet's Disease (BD) is a chronic systemic vasculitis of unknown etiology. Cutaneous lymphocyte antigen (CLA) has an important role in the migration of T cells into the inflamed skin.

Objective: Our aim was to investigate the relationship between CLA+T cell and clinical activity of BD.

Methods: A total of 80 Behçet patients and 20 healthy controls were involved in this study. BD patients were divided into four groups: ocular group genital ulcer group vessel/skin lesion group, inactive group. 10.000 cells in peripheral blood were used to assess the expression levels of CD3, CD4, CD8, CLA+CD4 and CLA+CD8 in lymphocytes with CLA/FITC clone HECA-452 kit.

Results: Erythrocyte sedimentation rate, C-reactive protein and lymphocyte CLA+CD8 levels were found to be higher, whereas CD4/CD8 and CLA+CD4/CLA+CD8 ratios were found lower in patients with BD when compared to controls.

Conclusions: CLA may be an important finding in the determination of etiopathogenesis, disease activity and prognosis of BD.

Keywords: Behçet's Disease, CLA, Immunology, Pathogenesis.

INTRODUCTION

Behçet's disease (BD) was firstly described by a Turkish dermatologist Hulusi Behçet in 1937 as triple symptom complex, characterized by oral aphtae (OA), genital ulcers (GU) and iridocyclitis with hypopyon [1]. Today, it has been understood that this syndrome is a chronic, inflammatory, multi-system vasculitis of both arteries and veins, gastrointestinal, pulmonary, cardiovascular and central nervous system involvements in addition to rheumatologic symptoms and signs [2].

The diagnosis of BD is made only with clinical symptoms, as there is no specific laboratory finding. It has been thought that environmental, immunological and genetic factors can be effective in BD with unknown etiopathogenesis. Immunological factors have been emphasized in recent years. Cellular and humoral response, increased neutrophil chemotaxis and T lymphocytes may play a role in the cutaneous form of BD [3]. T cell defect shows a change particularly in Th1/Th2 ratio, cytokine releases caused by Th1 immune response and tissue infiltration. Although the number of total T cells is reduced, it has been found that circulatory T cells are CD25 HLA-DR (+) active T cells, suggesting that T cells play an important role in the immunopathogenesis. There are increases in $CD4^+$-$CD16^+$, $CD4^+$-$CD56T^+$, $CD8^+$-$\alpha\beta^+$ and $CD8^+$-$CD11^+$ T helper cells in the active stage. The functional subgroup of T cells, namely $CD4CD45RA^+$ T helper cells induce $CD8^+$ suppressor/cytotoxic T lymphocytes and the studies have shown that $CD4CD45RA^+$ T cells are reduced. It is thought that the decrease in $CD4CD45RA^+$ cells may cause to the functional defect found in the suppressor T cells [3, 4].

Cutaneous lymphocyte antigen (CLA) is a carbohydrate epitope that is expressed in memory T cells. It facilitates binding of T cells to E-selectin, an adhesion molecule, and P-selectin [5]. The percentage of CLA+ T cells is approximately 90% at inflamed skin areas, whereas it is 15% in the circulation [6]. Therefore, it has been thought that CLA itself has a very important role in the migration of T cells into the inflamed skin areas [5]. The amount of CLA+ T cell subpopulations in the blood has been found higher in some dermatological diseases such as psoriasis and atopic dermatitis, which is affected by disease activity [5-9]. Mucocutaneous symptoms and signs are main findings of BD, which are the most frequently recurring lesions that lead up to the diagnosis of BD. Thus, it is thought that CLA+ T cells may be important in the clinical presentation of the disease.

This study aimed to establish the relationship between the percentage of CLA+ T cell subpopulations

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and disease activity or mucocutaneous involvement in BD.

MATERIALS AND METHODS

The study was conducted between August 2009 and August 2010. We were able to find 20 patients with ocular involvement. Therefore, we preferred each group to contain 20 subjects. As a result, a total 80 patients with BD and 20 age-and sex matched control subjects were included in this study. International Study Group criteria were used for the diagnosis of BD [10]. All participants were informed about the study and the study was conducted in accordance with the Declaration of Helsinki and local laws. The local ethics committee of the Erciyes University Faculty of Medicine approved the initial research proposal. The patients (80) were divided into 4 groups as follows:

Group 1 (ocular group): 20 patients with active ocular involvement with or without other systemic or mucocutaneous findings at the time of the study.

Group 2 (genital ulcer group): 20 patients with active genital lesion without ocular, vascular or other systemic involvement at the time of the study,

Group 3 (vessel/skin lesion group): 20 patients with active vessel/skin involvement including deep vein thrombosis, superficial thrombophlebitis, erythema nodosum and papulopustular or papulonodular lesions of the disease without ocular or genital lesion at the time of the study.

Group 4 (inactive group): 20 subjects with known diagnoses of BD but having no active disease manifestation at the time being.

The laboratory personnel were blinded to the clinical diagnosis and group of the subjects, matching each blood sample by letter coding. The same procedures were applied to all BD patients and control subjects. No patients with BD were interrupted the treatment including colchicine, systemic steroids, azathioprine, methotrexate, salazopyrine or topical medication during the study. 20 age and sex matched healthy individuals were employed as control group. After all subjects in each group gave their informed consent, they were carefully examined, and detailed medical histories were obtained. The onset of disease and the frequency of symptoms were recorded. All patients were underwent dermatological and ophthalmologic examinations. Pathergy test was performed in all BD patients. Complete blood count (CBC), biochemical parameters, ESR, ASO, CRP, expression levels of CD3, CD4, CD8 in peripheral blood leukocytes and expression levels of CLA+ CD4 and CLA+ CD8 in T lymphocytes were measured in all patients and control subjects. In each sample, 10.000 cells in peripheral blood were used to assess the expression levels of CD3, CD4, CD8, CLA+ CD4 and CLA+ CD8 in the lymphocytes. CLA/FITC clone HECA-452 kit and mouse IgG1 code no X40 were used for measurement of negative control.

15.0 SPSS for windows (Chicago) was used for statistical analysis by using chi-square and Kruskal-Wallis test. For multiple comparisons, Dunn's test and Tukey test were used. $p<0.05$ was considered as significant.

RESULTS

Of 80 BD patients, 43 (53.8%) were women and 37 (46.3%) were men. The mean age was 34.6±9.5 years (ranging 15 to 58 years). Both groups were similar for age and gender ($p >0.05$). The mean disease duration was found to be 5.8±6.3 years (range, 1 months-24 years). No significant difference was found between patients subgroups in term of mean age at the time of onset of the disease ($p>0.05$). Demographic findings of the patients with BD and controls are summarized in Table **1**.

Table 1: Demographic Properties of the Patients with BD and Controls

Groups		Age (year)			Disease duration (year)			Men		Women	
	n	Min.	Max.	Mean	Min.	Max.	Mean	n	%	n	%
Ocular	20	15	43	31,15	0,1	10	2,81	12	60	8	40
Genital ulcer	20	22	47	34,60	0,1	20	6,17	8	40	12	60
Vessel/skin lesion	20	21	50	33,20	0,1	21	5,46	12	60	8	40
Inactive	20	21	58	39,45	0,5	24	8,90	5	75	15	25
Control	20	23	50	34,75	-	-	-	10	50	10	50
p-value	-	-	-	>0,05	-	-	>0,05	-	>0,05	-	>0,05

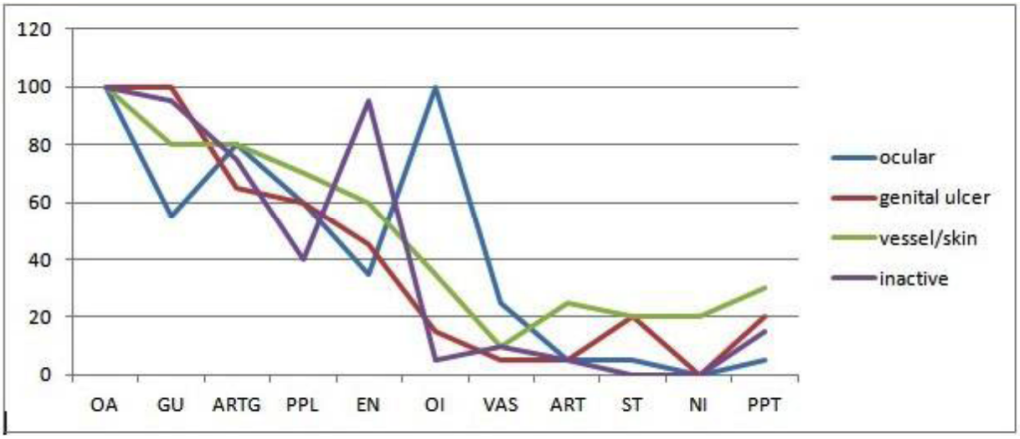

Figure 1: The frequencies of clinical findings of patients with BD. OA: oral aphtha, GU: genital ulcer, ARTG: arthralgia, PPL: papulo-pustular lesion, EN: erythema nodosum, OI: ocular involvement, VAS: major vascular involvement, ART: arthritis with radiological findings, ST: superficial thrombophlebitis, NI: neurological involvement, PPT: positive pathergy test.

When frequency of clinical finding was evaluated, the most common symptom was oral ulceration (80,100%), followed by genital ulceration (66, 82.4%), arthralgia (60, 75.0%), papulopustular/acneiform eruption (46, 57.5%), erythema nodosum (37, 46.3%), ocular involvement (31, 38.8%), major vascular involvement (10, 12.5%), arthritis with radiological findings (8, 10.0%), superficial thrombophlebitis (7, 8.8%), neurological involvement (4, 5%) and gastrointestinal involvement (1, 1.2%). Positive pathergy test was found in 14 (17.5%) of BD patients. The frequencies of clinical findings of patients with BD are shown in Figure **1**.

When all patients with BD were compared to control group, ESR, CRP and CLA+ CD8 levels in lymphocytes were found to be higher, whereas the ratio for CD4/CD8 and CLA+ CD4/CLA+ CD8 were lower ($p<0.05$ for each). No significant difference was detected in the percentage of CLA+ T cells by age in both patient and control groups ($p>0.05$). Hemoglobin values were found to be lower in group 1, 2 and 3 than control group, whereas white blood cell count and CRP levels were found to be significantly higher in group 1, 2 and 3 than inactive group. Despite being in normal limits, ALT values were higher in inactive group than the other patient groups with active disease ($p<0.05$). CLA+ CD8 levels in lymphocytes and ESR were found to be higher in patients with active lesions and inactive groups than those found in control subjects.

White blood cells (relative to inactive group) and CLA+ CD8 (relative to control group) values were higher in ocular group ($p<0.05$); ESR (relative to control group), ASO (relative to ocular group) and CRP values (relative to inactive group and control group) were higher in patients with active genital lesion ($p<0.05$); ESR (relative to control group) and CRP values (relative to inactive group and control group) were higher in patients with active vessel/skin involvement; and ESR (relative to control group) and ALT values (relative to genital lesion group) were higher in the inactive group ($p<0.05$ for each). Albumin values were found to be lower in patients with active vessel/skin involvement than all other subgroups, whereas CLA+ CD4/CLA+ CD8 T lymphocyte ratio was found to be lower in each 3 patient groups (vessel/skin lesion, ocular and inactive group) than control group ($p<0.05$ for each). Laboratory findings and CD4, CD8, CLA+CD4 ve CLA+CD8 levels of the patients with BD

Table 2: Laboratory Findings of the Patients with BD and Controls

Groups	Hb	WBC	ESR	ASO	CRP	Albumin	ALT
Ocular	13,2	8799	20	99	8,8	4,34	15
Genital ulcer	12,9	8406	42,4	187	28,3	4,33	13
Vessel/skin lesion	13,2	7680	37,7	140	25,1	4,11	20
Inactive	13,5	6394	25,3	150	5	4,38	23
Control	14	6897	11	135	3,3	4,23	19
p	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05

Table 3: The CD3, CD4, CD8, CLA+CD4 ve CLA+CD8 Levels of the Patients with BD and Controls

Groups	CD3 %	CD4 %	CD8 %	CLA+CD4 %	CLA+CD8 %	CD4/ CD8	CLA+CD4/ CLA+CD8
Ocular	76,46	42,36	27,23	2,25	0,58	1,68	5,39
Genital ulcer	80,28	43,84	28,43	2,58	0,40	1,71	7,51
Vessel/skin lesion	82,82	43,42	31,63	2,48	0,55	1,43	6,91
Inactive	76,94	41,01	27,02	2,87	0,53	1,55	6,57
Control	82,87	45,67	25,74	3,15	0,33	1,86	11,47
p	>0,05	>0,05	>0,05	>0,05	<0,05	<0,05	<0,05

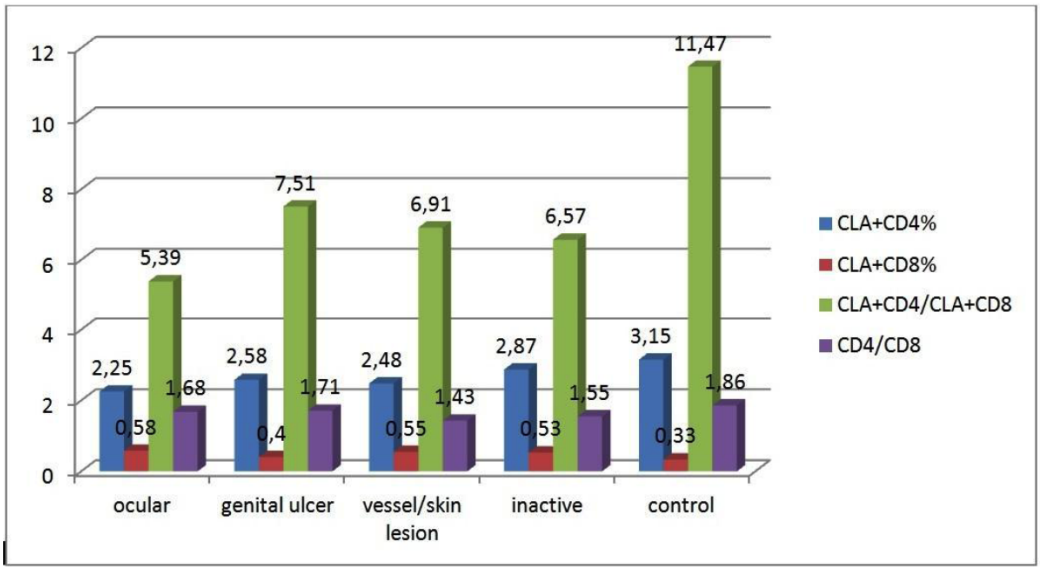

Figure 2: CD3, CLA+CD4, CLA+ CD8 expression levels with their proportions in T lymphocytes according to groups.

and controls are summarized in Tables **2** and **3** respectively. CD3, CLA+CD4, CLA+ CD8 expression levels with their proportions in T lymphocytes according to groups were shown in Figure **2**.

DISCUSSION

The incidence of BD was almost equal in both sexes with a male: female ratio of 0.86, though a male tendency was proposed in previous years [3, 4, 11, 12]. Although it is believed that gender has no effect on clinical course, it is usually thought that it is poorer in male patients [2, 3]. In this study, 53.3% of active group was consisted of men, whereas 46.6% was consisted of women. The disease is to be commonly seen between 20 and 40 years of life [1-4]. In the present study, mean age of the patients was found to be 35 years. In the patients of this study group, clinical findings were seen in similar ratios to those found in the literature. Although some reports accepted pathergy test as specific for BD, its positivity vary among geographical regions [3, 4]. In the present study, pathergy test positivity was detected in 17.5% of patients. In other studies of our clinic, positivity rate of pathergy test was found as 16.0-51.6% in adults, while 76.0% in pediatric patients [13-15]. Acute phase reactants are higher in active patients particularly in genital ulcer group as expected. Relatively higher level of ALT in inactive group may be associated with long term drug usage for treatment.

Actual pathology that causes BD is increased and prolonged inflammatory response with the presence of dense T cell infiltration at the inflammatory region, as it is evident from immunopathological evaluation of oral aphtae and the specimen of pathergy reaction. Presence of a strong Th1 polarization, its correlation with disease activity and effectiveness of cyclosporine that suppresses T cell functions in the treatment of BD are all other clues of T cell implications in this unique disorder [3, 4, 7]. In addition, it was shown that adenosine deaminase which has increased biological

activity during lymphocyte proliferation, maturation and differentiation, also exhibits increased activity during active episodes of BD suggesting that T cells (particularly Th1) are essential for BD pathogenesis [16]. It is assumed that T cells have hypersensitivity to various antigens and CD4+/CD8+ ratio is lower in BD as a result of decreased CD4+ T cells along with increased CD8+ T cells. The cause of decreased CD4+ T cells is the reduction of CD4+CD45RA+ T cells in the peripheral blood of patients with active disease [3]. In the present study, CD4+/CD8+ T cell ratio was found to be significantly lower in all patients with BD, when compared to control subjects. The migration of circulating T cells into the inflamed skin area is not a random process that is directed by CLA and several adhesion molecules, enzymes and chemokines during this process. CLA participates in complex molecular interactions between the circulating lymphocytes and the cutaneous vascular endothelium during lymphocyte migration. Multi-step process of leukocyte extravasation is also valid for the migration of CLA+ T cells into the skin.

CLA facilitates skin infiltration in T cell-mediated inflammatory skin diseases [17]. T cell dependent dermatologic diseases are psoriasis, atopic dermatitis, contact dermatitis, drug-induced delayed type cutaneous allergic reactions, cutaneous T cell lymphoma, vitiligo, bullous pemphigoid and alopecia areata, in which CLA+ T cell subpopulations have been found to be higher than healthy individuals that are altered by disease activity [5-9, 17]. In a study on psoriasis, a chronic inflammatory skin disease, it was found that there was a strong relationship between the severity of disease/PASI values and the percentage of circulating CLA+ CD8+ T cells and all of which are markedly higher than control subjects [9]. It is advocated that the bacterial super-antigen release due to streptococcal throat infections and IL-12 results in CLA+ T cells elevation in acute and guttat psoriasis [17, 18]. If it is thought that streptococci have a role in BD, CLA+ T cells may be important in its clinical presentation. In addition, an increase was found in the tissue and circulating CLA+ T cells of cutaneous T cell lymphoma and it was demonstrated that CLA+ T cells were found to be related to the extent of cutaneous lesions, suggesting for poor prognosis [17, 19-21].

In a study on 10 patients with atopic dermatitis, it has been reported that Calcitonin Gene-Related Peptide and neuropeptides induces cytokines expression by CLA+ memory T cells during the activation period of the disease [21, 22]. In another study on severe atopic dermatitis, it has been reported that no significant difference was detected among the percentages of CLA+ T cells when compared to control subjects. But still, it has been suggested that CLA may have a role in the development of atopic dermatitis and there may be additional surface proteins (unidentified), which might be effective on migration of T cells into the skin in atopic dermatitis. In the same study, an increase in the percentage of CLA+ T cells was detected in the non-atopic controls by age [5]. In the present study, no significant increase was detected in the percentage of CLA+ T cells by age in both patient and control groups.

We aimed to identify the role of CLA in the presentation and activation of BD, which demonstrates the skin orientation of T cell activation. This study has demonstrated for the first time that CLA+ CD8+ levels are higher in patients with BD. When comparison are made according to the subgroups, patients with active lesions, inactive patient group and ocular group have higher CLA+CD8 levels when compared with control subjects. Similarly, ratio of CLA+ CD4/CLA+ CD8 T lymphocyte was found to be lower in all patients with BD, as well as in active, inactive, ocular group and active vessel/skin disease when compared to controls. On the other hand, there were no significant inter or intra group differences in terms of CLA+ CD4+ T lymphocyte levels. Our findings demonstrated that CD8 and CLA+ CD8+ T lymphocyte levels were higher in the active disease. This may explain the reason for cumulation of findings at the mucocutaneous regions, when the disease is triggered anyway. However, given that there are several known and unknown mechanisms for CLA positivity, further studies are needed.

The number of patients and the controls can be considered as insufficient. The number of patients with ocular involvement was much less when compared with the other groups among our patients. That's why in each group there were 20 patients. It seems to be the limitation of the study.

In the present study, it was shown that CLA can be an important finding in the determination of etiopathogenesis, disease activity and prognosis of BD. In addition, it may play an important role in treatment approach in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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