The levels of intercellular adhesion molecule-1 in Behcet's disease

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Behget's disease is a chronic, relapsing systemic disease with unknown etiology. Intercellular adhesion molecule (ICAM-1) is relesaed from the cells of the inflammation site at transforming and developing state. clCAM-1 is considered as the circular form of ICAM-1 that formed by a proteolytic cleavage. It has been shown that plasma concentration of c-ICAM-1 is elevated in many inflammatory diseases. Plasma clCAM-1 concentrations were measured in order to determine the pathophysiologic role of clCAM-1 in the inflammatory events that seen in Behget's disease in this study. Comparing the mean plasma clCAM-1 concentrations in 44 Behget's disease patients (49.03+3.8 ng/ml) with 30 healthy controls (33.64±1.94 ng/ml) showed that difference was significantly high (p<0.01). Plasma concentration ofclCAM-1 in patients with active disease was significantly higher than patients in remission (p<0.001). Plasma concentrations of clCAM seemed to be in relation with the disease activity. [Turk J Med Res 1995, 13(4): 136-140]

Keywords: Behcet's disease, c-ICAM-1

Behcet's disease was first described by Hulusi Behcet in 1937. The disease has a chronic course with inflammatory relapses and its etiology is not clear (1,2). Almost all systemsare affected during the disease course (3,4,5). There are many contributing factors that may play role in the inflammatory process of Behcet's disease. Inter cellular adhesion molecule I (ICAM-1) is one of these factors. Results of some recent studies arouse this suspect (6-12). It has been shown that plasma levels of soluble form of ICAM-1 (c-ICAM-1) increases during several inflammatory conditions (13,14). From this point of view c-ICAM-1 level may be an indicator for acute inflammatory state in some specific conditions. In patients with active and inactive Behcet's disease, c-ICAM-1 levels were investigated in this study.

MATERIALS AND METHODS

This study was performed on 44 patients with Behcet's disease in Gulhane Military Medical Academy. Their diagnosis was made by using the proposed follow up criteria for Behget's disease. These criteria were proposed by International Study Group for Behget's disease in 1990. Thirty-six of the patients were males (82%) whereas 8 were females (18%). Age dispersion of the patients was among 17 to 64 and the mean age was 30,20 (SEM=1.49) disease duration was varied among 1 to 13 years.

Twenty-two males (73%), 8 females, 30 healthy persons were taken as controls. Age dispersion of controls was among 17 to 51 and the mean age was 32,73 (SEM-1.89).

The patients were accepted at active stage it they had at least two of the following symptoms: (1) Oral ulcer (2) genital ulcer (3) opthalmic lesion, plus an elevationr of CRP level or ESR (17). According to these criteria, the patients were separated into two groups either having active or inactive disease. Each group was separated into two subgroups if they had treatment or not. The results were expressed as mean ± SEM in Table 1 and 2.

clCAM-1 measurements: Venous blood samples were collected into EDTA contained test tubes (4 ml). Samples were centrifuged at 3000 rpm for 15 min. Then the plasma fractions of the samples were separated and stored at -15°C All samples were analyzed together.

1. All samples and immunoassay plates were taken to room temperature.

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2. x10 ul of patient's serum and 100 ul of conjugate were placed into the anti-human c-ICAM-1 covered wells.

3. Ptates were incubated at 37°C for 3 minutes. Then EIA plates were washed out five times by using an automatic washer. 100 ul of substrate solution (contains TMB ana H2O2) was added to each well and waited for 15 min at the room temperature. The ongoing reaction was stopped by adding NH2SO4 solution, then the concentrations in wells were assessed by using an automatic EIA reader at 492 nm wave length. Results were compared to a standard curve by usng microprocessor and expressed in numerical values.

Results of the analyze were evaluated by using Student's t test and Mann-Whitney U test.

RESULTS

Thirty patients had oral active aphts, 22 had active genital ulcers, 25 had active skin lesions, 8 had arthritis, 16 had eye involvement, and 7 had acute thrombophelebitis of 44 cases. Nine cases had positive family history. HLA typing carried out on 14 cases and 8 cases were positive for HLA B5 (Table 3).

cICAM-1 levels were found to be increase in patients with Behcet's disease as compared to controls ($p<0.01$). In active patients' group, cICAM-1 levels were significantly higher than controls ($p<0.001$) and inactive patients ($p<0.01$). There was no difference between two subgroups of active patients, according to cICAM-1 levels ($p>0.05$). There was statistical difference between active patients with therapy subgroup and inactive patient group ($p<0.05$), and active patients without therapy subgroup and inactive patients with therapy subgroup ($p<0.05$). There was also a significant difference between active patients without therapy subgroup and inactive patients without therapy subgroup.

Table 1. The mean levels of cICAM-1 in groups

	n	mean cICAM-1	SEM
Patients	44	49.03	3.86
Patients with active disease	24	69.14	2.38
Patients with inactive disease	20	24.89	3.11
Controls	30	33.64	1.94

Table 2. The mean levels of cICAM-1 in subgroups

	n	mean cICAM-1 value	SEM
Patients with active disease (under treatment)	9	65.17	3.97
Patients with active disease (without treatment)	15	71.52	2.90
Patients with inactive disease (under treatment)	11	33.42	3.48
Patients with inactive disease (without treatment)	9	14.47	1.70

DISCUSSION

ICAM-1 released from nonhematopoietic cells (like vascular endothelial cells, thymic epithelial cells, and fibroblasts), and hematopoietic cells (like tissue macrophages, stimulated T lymphocytes in tonsillar nodes, and B cells situated in germinal centres of Peyer plaques). Interaction between ICAM 1 and its integrin receptor LFA1 is an important suoject in immune control during inflammatory process and neoplastic transformation (15). ICAM 1 regulates the migration and adhesion of leukocytes by acting as a mediator (16).

As a member of immunoglobulin super gene family, it is well-know that ICAM 1 plays an important role in inflammatory states (1,17). ICAM 1 expression is rather restricted and most of the epithelial cells dose not express ICAM 1 under the normal conditions (18). Release of cICAM 1 is controlled by inflammatory cytokines as IL 1 and IFN (8). ICAM 1 effects infiltration and accumulation of T cells into the thyroid tissue in autoimmune thyroid diseases. It is reported that, macrophage-like synovial cells express ICAM-1 and inflammatory synovial tissue, synovial fibroblasts, tissue macrophages, and vascular endothelium release ICAM-1 molecules during rheumatoid arthritis (19). ICAM-1 takes part in the pathogenesis of adjuvant arthritis and in vivo use of anti ICAM-1 antibodies have strong inhibitory effect on the development arthritis (20).

Circulatory (soluble) form of ICAM-1 way recently defined. 82 KD weighted this molecular form is thought to be formed by proteolytic cleavage of ICAM-1. It has been shown that plasma levels of cICAM-1 increased during rejection phase of organ transplantation, Kawasaki disease, rheumatoid arthritis, asthma, malignant melanoma and leukocyte adhesion disorders and several'other diseases (21-23). There is no study in the literature about cICAM-1 levels in Behcet's disease. ICAM-1 and p2 integrin binding immunoglobulin like cell adhesion molecule deficiency was set up artifically in a study on mutant rats. These rats grew normally, but ICAM-1 surface distribution disappeared completely and inflammatory response decreased. According to these results, it has been suggested that inflammation may be prevented by decreasing the levels of ICAM-1 (24).

In patients with Hodgkin's disease, an increase in cICAM-1 level as compared to controls has been showed. It has been suggested that increase in ICAM-1 level may contribute to the adhesion mechanism of Hodgkin and Reed Stenberg cells, known as cytotoxic effectors. Thus, these cells may be protected from the

Table 3. Clinical and laboratory results of patients with Behcet's disease

No	Name	Age	Sex	Disease age	Oral ulcer	Genital ulcer	Skin findings	Arthritis	Eye findings	Familiy history	H LA B-5	Treat-ment	ESR	CRP	Other	ICAM
1	MY	30	M	13		+	+	+				C	25	12		65.3
2	HÖ	21	M	4	+	+			+			C	13	5		81.6
3	MA	27	M	1	+	+			+				62	96	Nevro Behçet	74.6
4	YG	35	M	10		+					+	CS	30	12		18.6
5	MA	25	M	4	+	+						C	6	5		12.7
6	SB	29	m	2			+					c	25	24		57.02
7	EY	23	M	2			+						36	24		68.6
8	HY	21	M	9	+	+	+	+					38	24		57.6
9	FS	28	M	4			+						20	5		8.25
10	AK	29	M	2	+	+	+					CS	48	55		94.8
11	MS	21	M	1	+	+	+	+					55	24	OBS(4+)	73.9
12	HD .	25	M	5	+	+	+						33	96		81.2
13	AE	54	M	7	+							c	10	5	IHD+Ht	14.9
14	PB	35	M	9									6	5		11.28
15	GA	36	F	r i	+							c	35	12		38.11
16	HA	43	F	11									12	5		19.3
17	NO	21	M	1		+	+		+				20	12		12.8
18	SO	39	M	6	+	+	+		+		+		65	12	Gastritis	26.11
19	MK	17	M	3			+					cs-c	25	5		37.11
20	EK	26	M	9	+		+		+			CS	10	12		39.2
21	MK	29	M	5	+		+		+			c	18	5		47.11
22	NÇ	44	F	6								c	10	5		40.3
23	2A	30	M	11*	+		+		+	+			20	12		81.03
24	MK	64	M	2	+			+					10	5	IHD+ gastritis	9.27
25	OA	39	M	10				+					20	12		17.11
26	NO	21	M	2		+			+							87.1
27	BA	29	M	10	+	+			+	+	+		65	24	GIT	58.3
28	i Y	21	M	2.	+		+	+	+		+	CS	20	5		63.1
29	YA	27	M	***H***									20	24		24.3
30	MG	26	F	3	+		+						23	6		75.1
31	SB	21	M	6		+	+				+	CS	5	5		57.3
32	AY	30	M	2		+	+						5	5		13.2
33	AO	25	M	6			+					c	5	5		29.1
34	ÖG	32	M	10	+		+	+		+	+	c	48	6		71.9
35	YK	21	M	1	+	+							8	10		63.2
36	GÇ	22	M	1	+	+	+						13	18		14.7
37	VK	35	M	3								c	4	5	Gastritis,HT	38.4
38	AE	21	M	1	+	+	+			+			50	37		56.7
39	AY	31	F	7	+	+					+	c	48	5		81.5
40	FA	51	F	4	+	+				+		c	24	5		61.6
41	ST	37	F	4	+			+		+		cs-c	13	5		47.1
42	MB	41	F	7								c	20	6		52.1
43	SA	21	M	6	+	+			+	+	+		3	5		70.3
44	MÖ	26	M	2	+	+				+		c	55	10		58.7

C: Colchicine, CS: Corticosteroid, GIT: Gastrointestinal Tract, HT: Hypertension, IHD: Ischemic heart disease, OBS: Occult blood in stool

influence of immune system and the spread of disease becomes easier (25,26).

It has been reported that serum cICAM-1 level increases in patients with bronchial asthma and allergic alveolitis. During the status asthmaticus, increase in cICAM-1 level becomes more clear (27,28). Application of anti ICAM-1 monoclonal antibodies during the allergic inflammations have decreased eosinophilic infiltration of the lungs and hyperresponsiveness of the airways in monkeys (29).

cICAM-1 and TNF receptor levels In cerebrospinal fluid were found to be increased during the acute attacks of multiple sclerosis as compared to the other neurologic disease. cICAM-1 and TNF receptor levels may be the immunologic markers for clinical activation in patients with multiple sclerosis. Serum cICAM-1 concentration also increases during viral encephalopathy (30,31).

cICAM-1 level was found to be increased in synovial fluid of patients with rheumatoid arthritis. Increase in cICAM-1 level was more significant in patients with vasculitis or pneumonitis. Increased cICAM-1 level was correlated with IgG RF, IgM RF, bSR and TNF-a. It has been suggested that cICAM-1 measurements may be an important marker in determining the stage of the inflammatory activity of rheumatoid arthritis patients (32).

In patients with Graves ophtalmopathy or psoriasis, cICAM-1 level was found to be increased as well (14,33). The increased release of ICAM-1 has been shown in the skin of systemic sclerosis patients that may contribute to the infiltration of pathogenic lymphocytes into the skin. Increase in cICAM-1 levels is proportional with the disease activity in patients with systemic sclerosis. cICAM-1 level measurements may be useful in determining the lymphocytic activation. Anti ICAM-1 monoclonal antibody therapy is being used in the treatment that of chronic inflammatory disease and the preliminary results in therapy-resistant rheumatoid arthritis patients is hopeful (34).

cICAM-1 level in Behcet's disease, where inflammation plays an important role in the pathogenesis, was investigated in this study. The results were concordant with the other studies. As compared to controls, cICAM-1 level was found to be increased in patients group. cICAM 1 level was also significantly increased in patients with active disease as compared to patients with inactive disea^ These results suggest, the use of cICAM-1 level measurements in Behcet's disease as an activation criteria.

cICAM-1 level was lower in inactive patients group as compared to the others (patients with active disease and controls). This condition may due to the use of antiinflammatory drugs. Several authors suggested that anti inflammatory effect of corticosteroids and colchicin is partly took place by decreasing cICAM-1 levels (10,35). The finding of cICAM-1 level was decreased in patients with inactive disease, supports the use of monoclonal anti ICAM-1 antibodies during the active stages of disease.

The preliminary results of the studies on monoclonal anti ICAM-1 antibodies is hopeful in the treatment of inflammatory diseases. Besides the positive effect of lowering ICAM-1 levels on the treatment of inflammatory diseases, it is still not clear how will it affect the other immune pathways.

It is interesting that cICAM-1 level was also found to be decreased in inactive patients without therapy subgroup. This may be the result of the antiinflammatory therapy that these patients had during the active period. These finding also suggests the importance of cICAM-1 in the development of the symptoms of Behcet's disease. An increase of cICAM 1 level occurs during the active stages in wide variety of diseases from encephalitis to systemic sclerosis. During the development of Behcet's disease, cICAM 1 seems to play a role during the late stage of inflammation rather than the early stages.

As a conclusion; there is a need for further studies about cICAM-1 on Behcet's disease that may contribute to the explanation of the pathogenesis, follow up and the treatment of the disease.

Behçet hastalığında intersellüler adhezyon molekülü 1 düzeyleri

Behçet hastalığı etiyolojisi bilinmeyen kronik, tekrarlayıcı sistemik bir hastalıktır. ICAM-1 inflamasyon hücrelerinin dönüşüm ve gelişim döneminde salınır. cICAM, ICAM-1'in proteolitik ayrışması ile ortaya çıkan dolaşan bir şekli olarak kabul edilir. ICAM-1'in plazma konsantrasyonunun birçok inflamatuar hastalıkta yükseldiği gösterilmiştir. Bu çalışmada, plazma cICAM-1 konsantrasyonları Behçet hastalarında görülen inflamatuar olaylarda cICAMOVin patofizyolojik rolünü göstermek amacı ile ölçüldü. Ortalama plazma cICAM-1 konsantrasyonları 44 Behçet hastası (49.03+3.8 ng/ml) ile 30 sağlıklı kontrol grubu (33.64+1.94 ng/ml) karşılaştırıldığında anlamlı bir fark görüldü (p<0.01). Aktif hastalıklı kişilerde cICAM-1'in plazma konsantrasyonu remisyonda olan hastalardan önemli derecede yüksekti (p<0.01). cICAM-1'in plazma konsantrasyonları hastalığın aktivitesi ile ilişkili olduğu görüldü.
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