Soluble interleukin-2 receptor levels in patients with Behcet's disease

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Behget's disease is a multi-systemic disorder involving different organs. The etiology of Behget's disease is still controversial and both viral and autoimmune mechanisms have been proposed by different investigators. A number of immunological variables have been found in patients with Behget's disease. We have investigated plasma levels of soluble interleukin-2 receptor (IL-2R) in patients with Behget's disease and in age and sex matched 30 healthy control subjects. We found that soluble IL-2R levels elevated in plasma of active group as compared with controls and inactive group. On the other hand, soluble IL-2R levels have been found elevated in plasma of inactive group as compared with control group, but not statistically significant. We suggest that IL-2R may play an important role in the pathogenesis of Behget's disease, so that the soluble forms of these molecules are elevated in plasma. [Turk J Med Res 1995, 13(5):195-197]

Key **Words:** Behcet's disease, Soluble IL-2 receptor

Behcet's disease is a systemic inflammatory disorder with an unknown etiology. Viral, genetic and environmental factors and autoimmunity are possible etiologic factors for Behcet's disease (1).

Interleukin-2 (IL-2) and IL-2R system has a central role in immune system, and is a better-known cytokine-cytokine receptor system than other cytokines in terms of their molecular biochemistries and functions. The factor inducing IL-2 production also stimulates IL-2R production in target cells (2,3). Soluble IL-2R (a-chain) forms by separating from the surface of cells carrying this receptor occurs in serum (4,5). It is reported that the increase in levels of plasma soluble IL-2R may have some relation with the activity degree of primary disease (6).

The presence of CD4+ lymphocyte accumulation was shown near retinal vessels in patients died of Behcet's disease. CD4+ T lymphocytes are responsible mainly in producing IL-2 (3).

On the histopathologic examination, lymphocyte and mononuclear cell infiltrations are prominent in lesions of Behcet's disease (7-10). These cells are among the ones mostly have CD44 molecule (11). Based on these data we aimed to investigate if there is any relation between soluble IL-2R levels and the clinic situation of patients with Behcet's disease.

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MATERIALS AND METHODS

This study was done with 33 cases diagnosed as Behget's disease in Giilhane Military Hospital (Departments of Internal Medicine, General Surgery, Dermatology, Eye Diseases, Physical Treatment and Rehabilitation). Three (9%) of the cases were females and 30 (91%) of them were males. The mean age was 30.45±1.7 years (range 17-64). The range of ages disease of the cases were 1-24 years. The diagnosis was determined according to the criteria of "International Behcet Working Group" (12). The cases were diveded into two groups as an active group (15 patients) and an inactive group (18 patients). Subjects with oral or genital ulcers, eye lesions, at least two active arthritis, high levels of C-reactive protein and erythrocyte sedimentation rate were accepted as active group (3).

As control group, 30 healthy subjects were chosen among the people applied to GATA Check-up Center. Three of them were females (10%) and 27 were males (90%). The mean age of the control group was 32.73±1.88 years (range 17-58).

Venous blood samples of cases were placed to tubes with EDTA and centrifugated at 3000 cycle /minute for 15 minutes. The plasma parts were separated from the rest and kept in deep-freeze (at -25 °C) until analyzing. After completing the collection, the plasma samples of patient and control groups were studied in Microbiology Department.

Soluble **IL**-2R **Analysis:** Soluble IL-2R (a subunit) concentrations were measured by Enzyme Immuno Assay method (EIA) (Bender Medsystems-Austria).

1. The plasma samples and kit were warmed room ^rnparature achieved.

2. Samples from patients and soluble IL-2R calibrators were studied by placing them into the double wells, By diluting the samples to the rate of 1/100, 10 ul sample ^ a sputintoeachwell.w3. 50 ul Antihuman soluble IL-2R conjugate of alka-lin phosphatase including patient samples was put into EIA plaque wells whose solid phase is covered with recombinant mica soluble IL-2R antibodies

4. These plaques were incubated 2 hours at 37 °C

5. After this incubation period the wells were washed 5 times by automatic EIA washer. Then a substrate solution containing 100 ml Orthophenilen diamine + pydrogen peroxide were put into the wells.

6. After incubating 15 minutes at dark and room temparature, the reaction was blocked by 1 N H2S04 stop solution.

7. Then plaques were measured at 450 nanometer wave length.

These results were evaluated by comparing with standart calibrators. Calibrators contain recombinant human soluble IL-2R with the amounts of 0.08, 0.5, 1.2, 2.4, 4 and 5 nanogram/ml. In the evaluation internal measurement difference and difference between measurements were found 1.3% and 1.9% respectively. The optic densities of soluble IL-2R calibrators were tested yvith graphics obtained by lineer-log regression analysis. Recording to these data, at least and at most measurable concentrations were found 0.08 ng/ml and 5 ng/ml, respectively. The concentrations higher than 5 ng/ml were measured by extending the regression curve.

Differences among variables were assessed by using Student's-t test and Mann-Whitney U test. Difference of sex distributions were compared with Chi-spuate test. Results are noted as mean ± standart error ox mean.

RESULTS

-The features and results of 33 patients with Behcet's disease and 30 healthy subjects were evaluated. There were no statistically significant difference of age and sex distribution between patient and control groups (p>0.05).

In the evaluation of plasma soluble IL-2R, the levels in disease group were higher than those in control group (p<0.001) (Table 1). Additionally, plasma soluble IL-2R levels in active patient group were sigpificantly higher than the levels in control group (p<0.001) (Table 2). Soluble IL-2R levels in active patient group were also higher than those in inactive patient group (p<0.05) (Table 3). Although the receptor lavels in inactive patient group were a little higher than the levels in control group, this difference was not statistically significant (p>0.05) (Table 4).

Table **1**. The comparison of patient and control groups

	Case Number	Soluble IL-2R	P value
Patient group	33	1.58±0.26	
Control group	30	0.73±0.11	<0.01

Table **2.** The comparison of active patient and control groups

	Case Number	Soluble IL-2R	P value
Active patient group	15	2.33±0.44	
Control group	30	0.73±0.11	<0.001

Table **3.** The comparison of active and inactive patient groups

	Case Number	Soluble IL-2R	P value
Active patient group	15	2.33±0.44	
Inactive patient group	18	0.96±0.21	<0.05

Table 4. The comparison of inactive patient group and control groups

	Number	Soluble IL-2R	P value
Inactive patient group	18	0.96±0.21	
Control group	30	0.73+0.11	>0.05

PISCUSSION

plasma soluble IL-2R levels in patient with Behcet's disease were studied in this article. The diagnosis of orr cases was determined according to the criteria of "International Behçet Working Group" (12). Patient yyere divided into two groups as active and inactive ones. The patients with oral and genital ulcers, eye lesions, at least two arthritis, high C-reactive protein and sedimentation levels were accepted as active (14). Fifteen of our cases were active and 18 were inactive.

Plasma soluble IL-2 receptor levels have a close relation to IL-2 production. IL-2 is a cytokin which is produced mainly in CD4+ T lymphocytes and it plays a major role in ummune system physiology. It affects the target cells by binding to IL-2 receptors on them. Mitogen or antigen presented by MHC-II molecule and produces IL-2 and also it is a trigger of IL-2 receptors occurence (15,16). The target cells of IL-2 are T and B lymphocytes, natural killer cells, monocytes, thymic stromal cells, olygodendricytes and endothelial cells (15). Occurence of soluble IL-2 receptors begin in target cells which is parallel to IL-2 receptor increase (4,5). Hence, the increase in serum IL-2 receptor levels is considered to be depend on immune system activation (6).

In some studies, it was reported that the levels of soluble IL-2 receptors were high in lymphoma and leukemia (16). Besides, serum IL-2 receptor levels were also high in patients with active rheumatoid arthritis (17), systemic lupus erythamatosis (18) and graft rejection after renal transplantation (19).

In this stduy we found that plasma soluble IL-2 receptor levels in patients with active Behcet's disease were higher than those in inactive patients and control subjects (p<0.01). Although plasma receptor levels in

inactive patients were higher than control subjects, it wasn't significant statistically (p>0.05). These results are comparable with those of the other studies related to Beliefs disease.

Hamzaoglu et al accepted as active patients who had 3 major symptoms according to Mason and Barnes criteria. They found that soluble IL-2 receptor levels in active patients were higher than those in control subjects and reported that result was evident especially in patients with symptoms of arthritis (20).

Akoglu et al evaluated their cases with Behcet's disease according to Mason and Barnes criteria and found high IL-2 receptor levels in those patients. They diveded the patients into four groups due to symptoms and repoted that the IL-2 receptor levels in the fourth group which had oral ulcers+genital ulcers+active eye lesions or arthritis+thrombophylebitis were higher than those in other groups or control group (21).

Our IL-2 receptor results are comparable with the results are comparable with the results of the other two studies. Using the last Behget's disease criteria (12) and getting the similar subject number in both the patient and control groups were differences of our study.

Benezra et al. determined the active patient group only by eye lesions. They found higher levels of soluble IL-2R in patients with eye lesions than those in patients without these lesions or control group (22).

There are other signs that IL-2 and IL-2R systems may be some relation with the pathogenesis of Behget's disease. Yamamoto et al. reported that lymphocytes obtained from patients with Behget's disease with active eye lesions responded stronger to S-antigen and IRBP-protein which have uveitogenic effect than those from control group. They added that this response was inhibited by monoclonal antibodies produced against CD4 and Class II MHC HLA-DR molecules (23). It is known that IL-2 is produced primarily by CD4+ lymphocytes (3).

Charteris et al. examined retinal vessels postmortem belonging to a patient died because.of the systemic involvement of Behget's disease. They found T lymphocyte infiltration which have CD4 and IL-2R markers in intramural and perivascular structures. They demonstrated HLA-DR antigens in retinal endotel and pigment cells of the same case. The working group showed that the density of those antigens in vascular structures of normal eyes was very low (24).

As a result, we consider that IL-2 and IL-2R systems have an effect in pathogenesis of Behget's disease and the levels of plasma soluble IL-2R increase due to this effect. It was demonstrated that although the levels in active patient group were higher than those in control group, there was no definite correlation between IL-2R levels and the disease activity in some patients.

Behçet hastalığında plazma solubl IL-2 reseptör düzeyleri

Behçet hastalığı çeşitli organları tutan multisistemik bir bozukluktur. Etiyolojisi hala tartışmalıdır. Etiyolojisinde vira! ve otoimmün mekanizmalar suçlanmaktadır. Behçet hastalığında çeşitli immünolojik değişiklikler olduğu tesbit edilmiştir. Biz de Behçet hastalarında plazma solubl IL-2 reseptör düzeylerini araştırdık. Kontrol grubu yaş ve cinsiyet açısından uyumlu 30 sağlıklı kişi ile oluşturuldu. Biz kontrol grubuna ve inaktif hasta grubuna kıyasla, aktif hasta grubunda solubl IL-2 reseptör düzeylerinin yükseldiğini tesbit ettik. İnaktif grup ile kontrol grubu karşılaştırıldığında ise inaktif hasta grubunda solubl IL-2 reseptörünün yüksek olduğu fakat yüksekliği istatistiki açıdan anlamlı olmadığı saptandı. Sonuç olarak biz Behçet hastalığının patogenezinde IL-2 reseptörlerinin önemli bir rol oynadığını ve bu role bağlı olarak, bu meloküllerin plazma düzeylerinin arttığı kanaatine vardık.
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