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Research Article

Comparison of Serum Levels of SIL-2R, IL-6, IL-10, TNF-α, CRP, ESR and Fibrinogen in Patients with Active and Inactive Behçet's Disease

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Abstract:

Behçet's disease (BD) is a chronic inflammatory illness that affects the entire body and is characterized by recurring episodes of oral aphthae, ocular and cutaneous lesions, and scrotal or vaginal ulcerations. The involvement of other organs and systems increases mortality in addition to the significant morbidity.

This study involved a total of 40 participants, 20 of whom were healthy controls and 20 of whom were patients (of the 20 Behçet's patients, 12 (60%) were in the active phase and 8 (40%) were in the inactive phase). There was no therapy being given to any of the 20 patients that would have affected their cytokine levels.

Only young men made up the patient and control groups because both early onset and male sex are signs of poor prognosis.

The ELISA method was used to measure the levels of serum cytokines. The statistical analysis of the derived numerical values employed the Mann-Whitney U Test.

We found a significant correlation between serum cytokine levels and classical acute phase markers in active Behçet's patients. ESR ($P < 0,001$), CRP ($P < 0,001$), fibrinogen ($P < 0,001$), IL-10 ($P < 0,001$), IL-6 ($P < 0,001$), SIL-2R ($P < 0,001$) and TNFα ($P < 0,001$).

There was no statistically significant difference in serum levels of classical acute phase markers ESR ($P = 0,746$), CRP ($P = 0,476$) and fibrinogen ($P = 0,940$) when inactive Behçet's patients and healthy controls were compared. However, serum levels of IL-10 ($P < 0,001$), IL-6 ($P = 0,001$), SIL-2R ($P < 0,001$) and TNFα ($P = 0,001$) were statistically different between inactive Behcet's patients and the control group.

Our research shows that even in the inactive phase, serum cytokine levels of Behçet's patients are much higher than the healthy control group. However, the levels of ESR, CRP and fibrinogen, which are classical acute phase markers, were found at normal levels in Behçet's patients in the inactive phase. These findings show that measurement of serum interleukin levels will enable us to take preventive measures for morbidity and mortality follow-up of Behçet's patients.

Key words: Behçet's Disease; SIL-2R; IL-6; IL-10; TNF-α

Introduction

Behçet's disease (BD) is a systemic, long-term inflammatory condition marked by recurring episodes of oral aphthae, cutaneous, ophthalmic, and scrotal or vaginal lesions. Vascular, articular, gastrointestinal, cardiac, pulmonary, renal, and neurologic dysfunction are all linked to Behçet's Disease. Despite the fact that the discoveries date back to Hippocrates, Turkish Professor Dr. Hulusi Behçet was the first to describe it as a distinct illness in 1937 [1, 2].

The "Silk Road," the Middle East, the Mediterranean coast, and Central Asia all have higher rates of BD. The disease's greatest documented prevalence, which ranges from 110 to 420 cases per 100,000 individuals, was originally noted in Turkey. BD is present practically everywhere in the world, despite the fact that regional prevalence varies greatly. The disease, which affects both sexes equally, is the most It often occurs between the ages of 20–40. Male sex and disease early onset as causes of poor prognosis accepted [2, 3].

The exact cause of BD is not known yet. The theory that BD is an aberrant immune response that is brought on by bacterial, viral, and other environmental antigens or autoantigens like heat shock proteins (HSPs) is the one that is now most widely supported [4–7].

Only clinical signs and symptoms can be used to make the diagnosis of BD because there isn't a universally recognized diagnostic laboratory test for the condition. The most widely used and widely acknowledged diagnostic criteria for Behçet's disease are the International Working Group for the criteria for BD (the presence of either typical eye lesions, typical cutaneous lesions, or a positive skin pathergy test in addition to oral ulcers and recurrent genital ulcers). Increased serum levels of biochemical factors including CRP and cytokines have been suggested as indicators of disease activity [8–12].

In order to better understand the etiopathogenesis of BD, numerous cytokines have been studied. Regarding the blood cytokine levels of BP in the active and inactive stages, these investigations found remarkably disparate findings.

Patients with BD have higher levels of proinflammatory cytokines, particularly when the disease is active. The enhanced inflammatory response is thought to be caused by this elevation, particularly those implicated in Th1-mediated inflammation, along with genetic susceptibility. The SlL-2 receptor is thought to contribute to local inflammation. (13,14). TNFα enhances all types of humoral and cellular immune response and acts synergistically with IL-6 [15, 16].

Since IL-10 inhibits the production of various cytokines, IL-10 was designated as “Cytokine Synthesis Inhibitory Factor” (CSIF) when it was first identified. However, IL-10 reduces inflammation by reducing nitric oxide synthase induction and toxic oxygen radicals and monocyte prostaglandin H synthase-2 (COX-2) in macrophages [17–20].

It has been reported that circulating levels of SIL-2R, TNFα, and IL-6 are increased in Behçet’s patients [21]. It has been suggested that the increase in serum SIL-2R, TNFα and IL-6 levels is more pronounced in active-stage Behçet's patients.

It was also found that the increase in serum SIL-2R and IL-6 levels was more pronounced in BP with complications; It has been reported that IL-6 levels increase in the cerebrospinal fluid of patients with neuro-Behçet's disease. There are also studies reporting that serum SIL-2R, IL-6, IL-4, IL-10 and TNFα levels in Behçet’s patients do not increase in both active and inactive periods. Serum cytokine levels and conventional acute phase reactants have also been compared in several research [22–27].

Material and Methods

This study was conducted to compare serum SIL-2R, IL-6, IL-10, TNF-α levels with serum CRP, ESR and fibrinogen levels in active and inactive young male Behçet's patients.

A total of 40 persons participated in this study, 20 of whom were Behçet’s Patients and 20 of whom were healthy controls. Only young men made up the patient and control groups because both early onset and male sex are signs of poor prognosis.

The patient and control groups were selected from among the young men who came for enlistment examinations. A control group was formed from those who did not have any health problems or complaints and had no pathological findings according to the results of physical examination and blood tests. Those who had a history of Behçet's disease and still had active findings and did not use drugs constituted the active patient group, and those without active findings constituted the inactive patient group. Those currently using any medication were not included in any group.

The patients with Behçet ranged in age from 20 to 30, with a mean age of 22.55 years. Their diagnoses were made in accordance with the clinical standards established by the "International Study Group". Twelve (12%) of the 20 Behçet’s patients were included in the trial during the inactive phase, while eighty (40%) were in the active phase. Patients with at least two of the oral ulcers, genital ulcers, eye lesions and active arthritis were considered active patients.

Healthy men in the same age range made up the control group. The control group's age range was 20 to 29, and the average age was 22.45 years.

Blood samples were taken from the patient and control groups in the morning, fasting, into sterile tubes for ESR, CRP, fibrinogen, cytokine levels (IL-6, IL-10, SIL-2R and TNFα). Blood samples taken for cytokine levels were centrifuged sterilely at 3000 rpm for 10 minutes and their serums were separated. The serums were placed in sterile tubes with patient names on them and stored in deep-freeze at -40°C.

Determination of Soluble Interleukin-2 Receptor: Serum SIL-2R level was measured using commercially available ELISA kit (CYTELISA; Cytimmunesciences, Paris, France).

1. The samples to be studied and the kit were brought to room temperature.
2. 100 µl of standard, patient sample and control materials were placed in each well.
3. 25 µl of Anti-Human SIL-2R monoclonal antibody was added to them.
4. In order to prevent evaporation, the plates were covered with Acetate Plate Sealer and incubated for 3 hours at room temperature.
5. The solutions in the wells were aspirated and emptied.
6. The wells were filled with 250 ml of washing solution, waited for 15–30 seconds and aspirated. This process was repeated 3–4 times. Plates were dried by inversion.
7. 50 µl of Anti-Rabbit conjugated Alkaline phosphatase was added to all wells.
8. The steps 5 and 6 were repeated.
9. 200 µl of the prepared coloring solution was added.
10. Plates were closed and incubated for 15 minutes at room temperature.
11. Plates were read at a wavelength of 492 nanometers.

Obtained results were evaluated by comparison with standard calibrators. The optical densities of the calibrators were analyzed with the graph obtained by performing linear-log regression analysis. The minimum SIL-2R level that the test can measure is 5 pg/ml. was in the form.

Tumor Necrosis Factor-α determination: Serum TNFα level was measured using commercially available ELISA kit (CYTELISA; Cytimmune sciences, Paris, France).

1. The samples to be studied and the kit were brought to room temperature.
2. 100 µl of standard, patient sample and control materials were placed in each well.
3. 25 µl of Anti-Human TNFα monoclonal antibody was added to them.
4. In order to prevent evaporation, the plates were covered with Acetate Plate Sealer and incubated for 3 hours at room temperature.
5. The solutions in the wells were aspirated and emptied.
6. The wells were filled with 250 ml of washing solution, waited for 15–30 seconds and aspirated. This process was repeated 3–4 times. Plates were dried by inversion.
7. 50 µl of Anti-Rabbit Conjugated Alkaline Phosphatase was Added to all wells.
8. The steps 5 and 6 were repeated.
9. 200 µl of the prepared coloring solution was added.

10. Plates were closed and incubated for 15 minutes at room temperature.
11. Plates were read at a wavelength of 492 nanometers.

Obtained results were evaluated by comparison with standard calibrators. The optical densities of the calibrators were analyzed with the graph obtained by performing linear-log regression analysis. The minimum TNFα level that the test can measure is 5 pg/ml. was in the form.

Interleukin-6 determination: Serum IL-6 level was measured using commercially available ELISA kit (CYTELISA; The Cytoscreen, Paris, France).

1. The samples to be studied and the kit were brought to room temperature.
2. 50 µl of standard, patient sample and control materials were placed in each well.
3. 50 µl of Anti-IL-6 antibody was added to them.
4. Plates were covered and incubated for 1.5 hours at room temperature.
5. The solutions in the wells were aspirated and emptied.
6. The wells were filled with washing solution, waited for 15–30 seconds and aspirated. This process was repeated 3–4 times. Plates were dried by inversion.
7. 100 µl of Streptavidin was added to all wells.
8. Plates were closed and incubated for 30 minutes at room temperature.
9. The steps 5 and 6 were repeated.
10. 100 µl of coloring solution was added.
11. Plates were sealed and incubated at room temperature for 30 minutes in the dark.
12. Plates were read at a wavelength of 450 nanometers.

Obtained results were evaluated by comparison with standard calibrators. The optical densities of the calibrators were analyzed with the graph obtained by performing linear-log regression analysis. The minimum IL-6 level that the test can measure is 10 pg/ml. was in the form.

Interleukin-10 determination: Serum IL-10 level was measured using commercially available ELISA kit (CYTELISA; The Cytoscreen, Paris, France).

1. The samples to be studied and the kit were brought to room temperature.
2. 100 µl of standard, patient sample and control materials were placed in each well.
3. 50 µl of Anti-IL-10 antibody was added to them.
4. Plates were covered and incubated at 37°C for 2 hours.
5. The solutions in the wells are aspirated and emptied.
6. The wells were filled with washing solution, waited for 15–30 seconds and aspirated. This process was repeated 3–4 times. Plates were dried by inversion.
7. 100 µl of Streptavidin was added to all wells.
8. Plates were closed and incubated at 37°C for 45 minutes.
9. The steps 5 and 6 were repeated.
10. 100 µl of coloring solution was added.
11. Plates were sealed and incubated at room temperature for 30 minutes in the dark.
12. Plates were read at a wavelength of 450 nanometers.

Obtained results were evaluated by comparison with standard calibrators. The optical densities of the calibrators were analyzed with the graph obtained by performing linear-log regression analysis. The minimum IL-10 level that the test can measure is 5 pg/ml. was in the form.

In the statistical calculations of the numerical values obtained, the "Significance Test of the Difference Between Two Means" or the "Mann-Whitney U Test" was used in accordance with the number of cases. Results are given as arithmetic mean ± standard error. Values of $P \leq 0,05$ were considered statistically significant.

Table 1 summarizes the results of the active (12 patients) BP, while Table 2 summarizes the results of the inactive (8 patients) BP. Table 3 summarizes the results of the control group.

	Lower value	Upper value	Average	Standard error	Standard deviation
Age (Year)	20	30	22,55	1,20	2,71
ESR (mm/h)	20	105	66,25	6,50	22,52
CRP (mg/dl)	11,00	47,00	25,25	3,36	11,63
Fibrinogen (mg/dl)	386	585	474,08	16,93	58,64
IL-10 (pg/ml)	93,40	500,00	257,78	45,54	157,76
IL-6 (pg/ml)	7,80	93,40	32,96	7,18	24,90
SIL-2R (pg/ml)	7,80	125,00	34,62	9,27	32,14
TNF-α (pg/ml)	7,80	62,50	47,36	5,53	19,17

Table 1: Findings of Active Behçet's Patients (n = 12)

	Lower value	Upper value	Average	Standard error error	Standard deviation
Age (Year)	20	30	22,60	1,18	2,68
ESR (mm/h)	3	20	12,38	4,51	8,40
CRP (mg/dl)	5,90	7,00	6,03	0,14	0,38

	Lower value	Upper value	Average	Standard error error	Standard deviation
Fibrinogen (mg/dl)	265	622	391,50	38,56	109,05
IL-10 (pg/ml)	15,20	125,00	29,80	13,62	38,54
IL-6 (pg/ml)	7,80	22,40	11,45	2,38	6,75
SIL-2R (pg/ml)	7,80	15,60	10,72	1,42	4,03
TNF-α (pg/ml)	7,80	42,50	14,08	4,24	12,01

Table 2: Findings of Inactive Behçet's Patients (n = 8)

	Lower value	Upper value	Average	Standard error	Standard deviation
Age (Year)	20	29	22,45	1,30	2,61
ESR (mm/h)	2	20	7,65	3,25	4,48
CRP (mg/dl)	5,90	5,90	5,90	0,28	0,00
Fibrinogen (mg/dl)	191	382	288,20	21,45	47,05
IL-10 (pg/ml)	7,80	15,60	8,58	1,38	2,40
IL-6 (pg/ml)	7,80	15,60	10,14	1,45	3,66
SIL-2R (pg/ml)	7,80	15,60	9,36	1,40	3,20
TNF-α (pg/ml)	15,60	42,50	19,63	8,42	9,85

Table 3: Findings of the control group (n = 20)

Comparison the serum levels of conventional acute phase markers of active and inactive BP with control group is showed in Figure 1.

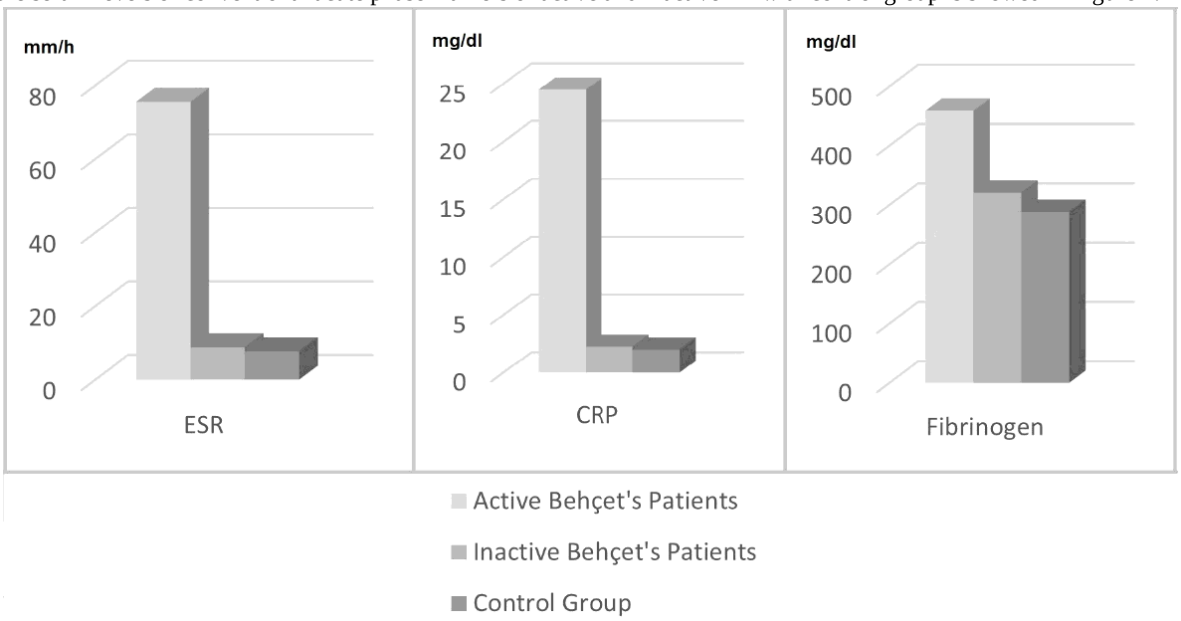

Figure 1: Comparison of serum levels of classical acute phase markers

Comparison the serum levels of SIL2-R, IL-6, IL-10 and TNFα of active and inactive BP with control group is showed in Figure 2.

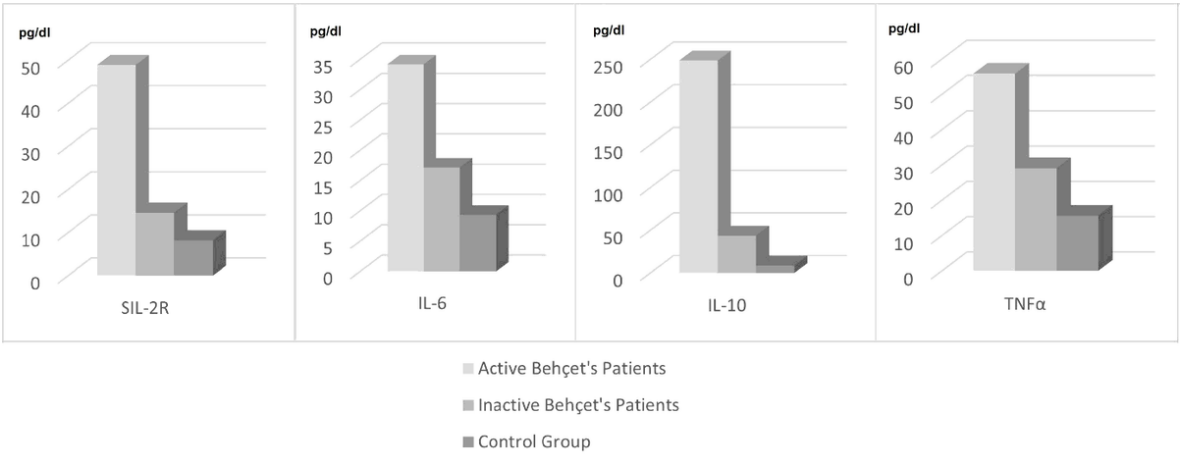

Figure 2: Comparison of serum levels of cytokines

Serum levels of ESR (P < 0,001), CRP (P < 0,001), fibrinogen (P < 0,001), IL-10 (P < 0,001), IL-6 (P < 0,001), SIL-2R (P < 0,001), and TNFα (P < 0,001) were statistically different between active and inactive Behçet's Patients (Table 4, Fig. 1).

	ESR	CRP	Fibrinogen	IL-10	IL-6	SIL-2R	TNF-α
Mann-Whitney U	0,500	0,000	18,500	10,500	22,500	14,500	8,000
Wilcoxon W	36,500	36,00	54,500	46,500	58,500	50,500	44,000
Z value	-3,672	-3,714	-2,279	-2,971	-2,052	-2,777	-3,304
Asymp. Sig. [2- tailed]	0,000	0,000	0,023	0,003	0,017	0,005	0,001
P value	0,000	0,000	0,020	0,002	0,047	0,007	0,001

Table 4: Comparison of Findings of Active-Inactive Behçet's Patients

Serum levels of ESR (P < 0,001), CRP (P < 0,001), fibrinogen (P < 0,001), IL-10 (P < 0,021), IL-6 (P < 0,001), SIL-2R (P < 0,001), and TNFα (P < 0,001) were statistically different between active Behçet's Patients and the control group (Table 5, Figure. 1).

	ESR	CRP	Fibrinogen	IL-10	IL-6	SIL-2R	TNF-α
Mann-Whitney U	0,500	0,000	13,500	2,000	24,000	11,500	6,000
Wilcoxon W	210,500	210,00	225,500	212,00	234,00	221,500	216,000
Z value	-4,668	-4,729	-4,147	-5,076	-4,139	-4,880	-4,886
Asymp. Sig. [2- tailed]	0,000	0,000	0,000	0,000	0,000	0,000	0,000
P value	0,000	0,000	0,000	0,000	0,000	0,000	0,000

Table 5: Comparison of Active Behçet's Patients-Control Group.

Serum levels of ESR (P = 0,746), CRP (P = 0,746), and fibrinogen (P = 0,940)) were not statistically different between inactive Behçet's Patients and the control group (Table 6, Fig. 1).

	ESR	CRP	Fibrinogen	IL-10	IL-6	SIL-2R	TNF-α
Mann-Whitney U	73,500	73,000	78,00	10,000	20,000	14,000	20,000
Wilcoxon W	109,500	283,00	114,00	220,000	223,000	224,000	223,000
Z value	-0,334	-0,373	-0,102	-4,170	-3,517	-4,287	-4,291
Asymp. Sig. [2- tailed]	0,738	0,709	0,919	0,000	0,000	0,000	0,000
P value	0,746	0,746	0,940	0,000	0,001	0,000	0,001

Table 6: Comparison of Inactive Behçet's Patients-Control Group

Serum levels of IL-10 ($P < 0,001$), IL-6 ($P = 0,001$), SIL-2R ($P < 0,001$), and TNFα ($P = 0,001$) were statistically different between inactive Behçet's patients and the control group (Fig. 2).

Discussion

The exact cause of BD is not yet known. The theory that BD is an aberrant immune response in people who are genetically predisposed to the disease that is brought on by environmental antigens or autoantigens like heat shock proteins (HSPs) is currently the one with the greatest evidence [28, 29].

Patients with BD have higher levels of proinflammatory cytokines, particularly when the disease is active. The enhanced inflammatory response is thought to be caused by this elevation, particularly those implicated in Th1-mediated inflammation, along with genetic susceptibility [28–30].

The literature is replete with studies examining IL-10 serum levels. Sadeghi et al. discovered no significant difference in serum levels of IL-10 between Behçet's patients and a control group, according to Aridogan et al. and Guenane et al [31, 32].

However, in our study, we found a substantial difference in serum IL-10 levels between patients with active BD and the control group, as well as between patients with inactive BD and those who did not have it.

Acute phase reactant CRP is known to be increased in a range of inflammatory diseases. The CRP level may potentially be a sign of tissue damage caused by the immune system. In our investigation, a substantial difference was found between the CRP levels of active BP and the control group.

Numerous studies examining CRP serum levels have been published, and several of these studies (e.g. Karada et al., Müftüolu et al., Balta et al., Bekpınar et al., Adam et al.) reveal that serum CRP levels are elevated in individuals with active BD [33–35].

In several disorders, including Behçet's patients in the active phase, investigations have shown that ESR and CRP rise as acute phase reactants [36, 37]. Most active BD symptoms are linked to both ESR and CRP. They can be applied to Behçet's patients to forecast disease activity and active vascular symptoms [37].

In addition to serving as a marker of inflammation, fibrinogen, an acute phase reactant, is essential for the production of clots in both the fibrin network and platelet aggregation. In order to understand the vascular involvement and to demonstrate the disease activity, the increase in fibrinogen in Behçet's illness is crucial [38].

These cytokines were chosen because IL-6, SIL-2R and TNFα are proinflammatory; IL-10 is known as an anti-inflammatory cytokine and there is more research and knowledge about these cytokines. In addition, the increase in serum levels of these cytokines in many inflammatory diseases suggests that they will be effective both in the pathogenesis of the disease and in the evaluation of disease activity, as well as in the treatment.

This study can be criticized in two aspects: One of them is the low number of participants, and the other is that the results were obtained in this way because there may be organ involvement in Behçet's patients in the inactive period (no real inactive period). However, it is not easy to find young male patients who have never received treatment. Yes, it would be better if more young men participated in this study. Perhaps in the future, such a study will be conducted with a large number of participants.

Secondly, it is important that although the classical acute phase markers are normal, cytokines are high in these patients, even though Behçet's patients in the inactive period are not really in the inactive period due to organ involvement. Because detecting a condition that cannot be detected with conventional acute phase markers with cytokine levels is very important for morbidity and mortality follow-up.

Another question that may come to mind: Why was IL-10, an anti-inflammatory cytokine, found to be high, as well as high levels of proinflammatory cytokines in Behçet's patients in the inactive period? In response to this: Perhaps the inactive period of these patients is due to this anti-inflammatory cytokine. IL-10, which is an anti-inflammatory cytokine, increases more because proinflammatory cytokines increase more in the active period, and IL-10 increases less in the inactive period, since the increase in proinflammatory cytokines is less.

Conclusions

The early onset and male sex of BD are factors that are thought to be detrimental to prognosis. Along with significant oral, vaginal, and cutaneous ulceration as well as eye involvement, other organs and systems involvement increases mortality.

Our study is significant because it only looked at young male Behçet patients. Additionally, no other study has compared cytokines with classical acute phase indicators in young male BD patients who are active and inactive.

Our findings showed that even in the inactive phase, serum cytokine levels of Behçet's patients are much higher than the healthy control group. However, the levels of ESR, CRP and Fibrinogen, which are other acute phase markers, were found at normal levels in Behçet's patients in the inactive phase.

These findings show that measurement of serum interleukin levels will enable us to take preventive measures for morbidity and mortality follow-up of Behçet's patients.

Declarations

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Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

This article is derived from the specialty thesis in medicine. Therefore, the only author is Ali Osman Avcı, MD. Those who contributed during the thesis work are included in the acknowledgment section.

Ethics approval and consent to participate

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University Gulhane Military Medical Academy (2020/27).

Patient consent for publication

The author affirms that human research participants provided informed consent for publication.

Competing interests

The authors declare that they have no competing interests.

Additional Declarations

No competing interests reported.

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