Serum Levels of Interleukin-36 Alpha and Interleukin-36 Receptor Antagonist In Behcet’s Syndrome

The median IL-36α level was 0.11 ng/ml in the BS group and 0.09 ng/ml in the HC group (p = 0.058). The mean IL-36Ra level was 13.62 pg/ml in the BS group and 13.26 pg/ml in the HC group (p = 0.348). Serum IL-36Ra levels of the active group were significantly higher (p = 0.037). Patients with oral ulcers and central nervous system involvement had higher serum IL36Ra levels. In the BS group, a positive correlation was found between serum IL-36Ra and CRP. In a multivariate analysis, the IL-36Ra level (OR = 1.067; 95 % CI = 1.001–1.137; p = 0.045) was independently associated with disease activity.

Conclusion According to these findings, it is not clear whether such a slight difference is clinically significant, but they suggest that the IL-36 cytokine family may play a role in the course of the disease.

ZUSAMMENFASSUNG

Hintergrund Das Behcet-Syndrom (BS) ist eine Form der systemischen Vaskulitiden. Ziel dieser Studie war es, die Serumspiegel von IL-36α und IL36Ra bei Patienten mit BS zu untersuchen.

Material und Methoden Insgesamt 80 Probanden (60 BS-Patienten und 20 gesunde Kontrollpersonen [KP]) wurden in die Studie eingeschlossen.

Ergebnisse Der mittlere IL-36α-Spiegel betrug im BS 0,11 ng/ml und im KP 0,09 ng/ml (p = 0,058). Der mittlere IL36Ra-Spiegel betrug 13,62 pg/ml im BS und 13,26 pg/ml im KP (p = 0,348). Der IL-36Ra Serumspiegel der aktiven Gruppe waren signifikant höher (p = 0,037). Patienten mit oralen Geschwüren und Beteiligung des zentralen Nervensystems hatten höhere IL-36Ra-Serumspiegel. In der BS Gruppe wurde eine positive Korrelation zwischen IL-36Ra und CRP im Serum gefunden. In der multivariaten Analyse war der IL-36Ra-Spiegel (OR = 1067; 95 % CI = 1001–1137; p = 0045) unabhängig mit der Krankheitsaktivität assoziiert.

Schlussfolgerung Nach diesen Erkenntnissen ist nicht klar, ob ein derart geringfügiger Unterschied eine klinische Bedeutung hat. Die Daten legen jedoch nahe, dass die IL-36-Zytokin familie möglicherweise eine Rolle im Krankheitsverlauf spielt.
Introduction

Behçet’s syndrome (BS), that affect multiple systems in a chronic course with exacerbations and remissions, is a vasculitic disease. BS is characterized by recurrent oral, genital ulcers, uveitis, arthrits and vascular gastrointestinal, neurological, pulmonary and cardiac systems involvement [1].

The etiopathogenesis of the syndrome is still unclear. Inflammation, infection, genetic factors, and complex changes in innate and acquired immunity play a role in the pathogenesis [2]. Previous studies reported higher serum levels of inflammatory cytokines in patients with Behçet’s syndrome [3, 4]. Mostly, Interleukin (IL)-1 family cytokines are investigated to better understand the disease pathogenesis. The new members of the IL-1 cytokine family are IL-36α, IL-36β, IL-36γ, and IL-36 receptor antagonists (Ra). These cytokines are located on human chromosome 2 like most of the other IL-1 family members [5]. IL-36 plays a vital role in innate and acquired immunity. IL-36 stimulates dendritic cells from the host cells of innate and acquired immunity. Human dendritic cells also induce IL-1, IL-6, IL-12, IL-18 and IL-23 secretion when stimulated with IL-36 and increase CD83, CD86, and MHC II expression on the cell surface. In addition, IL-36 stimulation increases allogeneic T cell proliferation and IFN-γ release [6–8]. Previous studies showed that IL-36 may important in the pathogenesis of inflammatory diseases such as psoriasis, rheumatoid arthritis (RA), Sjögren’s syndrome, systemic lupus erythematosus and inflammatory bowel diseases [9–11]. IL-36Ra acts as a natural antagonist of IL-36α by binding to the IL-36 receptor. Deficiency of the interleukin-36 receptor antagonist may lead to an inflammatory skin condition known as generalized pustular psoriasis [12].

This study aimed to investigate the levels of serum IL-36α and IL-36Ra in Turkish patients with BS and to determine its correlation with disease activity and clinical manifestations.

Materials and Methods

Participants

Eighty patients were included in the study and 60 of them were diagnosed with Behçet’s Disease according to the “International Study Group for Behçet’s Disease”. Behçet’s patients were followed up at Ankara University Faculty of Medicine, multidisciplinary BS diagnosis and treatment unit between 2015 and 2017. Twenty age and sex matched healthy persons who had no acute or chronic diseases were enrolled to serve as the healthy control (HC) group. Medical histories of all HCs were recorded and venous blood samples were taken after systemic and rheumatological assessment. The demographic data and symptoms of the patients were questioned, and the systemic involvement since the onset of the disease was evaluated. Active disease was defined as the presence of oral ulcers, and at least two of the clinical findings (skin lesions, genital ulcers, active arthritis and recent vascular, ocular or neurological involvement) found during the taking of blood samples. BS was also accepted as active if the high erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were detected with clinical symptoms despite current treatment [13]. The Ethical Committee of Ankara University Faculty of Medicine approved research protocol (No: 08–346–16). Each participant provided written informed consent.

Assay for IL-36α and IL-36Ra

Serum IL-36α and IL-36Ra levels were measured by sandwich enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer’s instructions (My BioSource, San Diego, CA, USA). The obtained venous blood samples for IL36α and IL-36Ra measurement were stored at − 80 °C after the centrifugation process. The IL-36α kit sensitivity was < 0.094 ng/ml and the IL-36Ra kit sensitivity was 1.95 pg/ml.

Statistical analysis

Visual and analytical procedures were used to evaluate whether numerical variables were normally distributed. T-test or Mann Whitney U test was applied according to whether the variables were normally distributed. Chi-square test for categorical variables was used to compare possible associated factors. Spearman correlation constants were used when the relationship between variables was examined. Multivariate analyses was performed using a backward logistic regression model to predict disease activity. Possible factors that were indicated in univariate analyses were entered into the logistic regression analyses in order to determine the independent correlates for disease activity. Hosmer-Lemeshow goodness of fit statistics were used to assess model fit. All data were analyzed using IBM SPSS Statistics 23. p values <0.05 were considered statistically significant.

Results

Sixty patients with BS and twenty age and sex-matched healthy controls were included in the study. Thirty-one of the patients had active disease (51.7 %). The median age was 42 (18–65) in all BS patients and 41 (21–64) in the HCs. Median disease duration of BS patients was 7.5 (1–28) years. Demographic and clinical parameters are shown in Table 1.

Median serum IL-36α level was 0.1102 (0.09–10.08) ng/ml in the BS group and 0.0940 (0.09–0.42) ng/ml in the HC group (p = 0.058). Serum IL-36α levels were similar both in patients with active and inactive disease (median[IQR 0.09–10.08 ng/ml in the active group and 0.0940 (0.09–0.42) ng/ml in the inactive group, respectively; p = 0.8). There was no statistically significant difference between serum IL-36α levels in active versus control and inactive versus control groups (p = 0.069 and p = 0.1, respectively) (Fig. 1).

Mean serum IL-36Ra level was found to be 15.3 ± 9.3 pg/ml in the BS group and 17.90 ± 13 pg/ml in the HC group (p = 0.348). When compared with the inactive group, serum IL-36Ra levels of the active group were significantly higher (17.80 ± 10.63 pg/ml vs 12.77 ± 7.12 pg/ml, respectively, p = 0.037). There was no statistically significant difference between serum IL-36α levels in active versus control and inactive versus control groups (p = 0.069 and p = 0.082) (Fig. 2).

Serum IL-36Ra levels in patients with oral ulcers was significantly higher from without oral ulcer (p = 0.018). Also, serum IL-36Ra levels were higher in patients with central nervous system (CNS) involvement than those without neurological involvement (24.45 ± 11.58 pg/ml vs 14.45 ± 8.71 pg/ml, p = 0.011). No statis-
In our study we found that serum IL-36Ra levels were slightly higher in the active BS group compared to the HCs and the inactive BS group. Despite being statistically insignificant we still believe this finding should be noted due to its concordance with previous studies suggesting that inflammation in BS is mostly mediated by IL-1 cytokines [18]. But the more considerable and also statistically significant result of our study was the detection of higher IL-36Ra levels in active BS group. We presume this elevation may be a healthy response that aims to limit the inflammation. Due to lack of detailed studies about IL-36Ra levels in BS pathogenesis we can not support our presumption with existing literature. But fortunately, in recent years several comprehensive reviews regarding regulation, expression and immune functions of IL-36 family cytokines were published [11, 19, 20]. Although the certain mechanisms of these cytokines in the pathogenesis of inflammatory conditions are not revealed yet, the one thing all authors agreed on was the anti-inflammatory capability of IL-36Ra which uses a similar mechanism with IL-1Ra for antagonizing IL-1α. Anakinra, drug the recombinant IL-1 receptor antagonist, is used in rheumatoid arthritis and deficiency of IL-1 Receptor Antagonist (DIRA) [21]. Since the recognition of the deficiency of the IL-36-receptor antagonist (DIRA), characterized by an aggressive form of psoriasis, numerous reports have been published suggesting that drugs targeting IL-1 pathway...
may be beneficial in treatment [22–24]. However, IL-36Ra is currently not used as therapeutics on the clinical use.

IL-36α, IL-36β, IL-36γ, and IL-36Ra are generally produced by keratinocytes in the skin. IL-36 cytokines are important in infectious and inflammatory skin diseases, especially psoriasis [25]. In patients with psoriasis, IL-36Ra, IL-36α, IL-36β and IL-36γ levels have been shown to be higher than the control group [26]. We didn’t find any differences in serum IL-36α and IL-36Ra levels in Behcet’s patients with cutaneous involvement compared to without involvement. In our study, only serum IL-36Ra levels were higher in patients with oral ulcers than without.

IL-36 is an also important factor for arthritis. Frey S et al. found that synovium IL-36α levels were higher in patients with psoriasis and RA than osteoarthritis. In this study, there was no difference between the three groups of IL-36 receptor and IL-36 Ra expression [27]. In patients with psoriasis and rheumatoid arthritis, IL-36α, γ and IL-36Ra levels were elevated and correlated with IL-1β, but IL-36 β levels were elevated only in patients with RA [28]. In another study; IL-36 receptor is expressed in human synovial fibroblast and human articular chondrocytes but there was no increase in IL36β levels in the inflamed human and mouse joints. Serum IL-36β levels were also similar in healthy controls with rheumatoid arthritis, osteoarthritis [29]. In another study which compared RA and psoriatic arthritis showed that IL-36α is expressed at similar levels in the synovium while psoriatic arthritis synovium expressed less IL-36Ra compared with RA. And researchers speculated that this result may be associated with decreased sensitivity to DMARDs in psoriatic arthritis patients. This study also demonstrated significantly higher plasma IL-36α levels in RA patients compared with psoriatic arthritis patients, but no difference for the IL-36RA levels [30]. In the TNF-induced arthritic mice, IL-36α and IL-36 receptor levels were higher than normal mice and IL-36Ra levels were found to be similar. No changes in disease severity were observed after administration of anti-IL-36 receptor antagonist to TNF-induced arthritic mice [31]. As a result, it was thought that IL-36 was not a mediator of synovial inflammation [32]. In our study, we found that serum IL-36α and IL-36Ra levels were similar in patients with and without arthritis.

In a brain cell study, IL-1Rrp2 (IL-36 receptor) was expressed in astrocytes and microglia, but this expression was not observed in neurons [33]. Previous studies found that IL-36 levels elevated in patients with multiple sclerosis and neuromyelitis optica spectrum disorder patients [21]. Karumbaiah L. et al. found that in an animal model in which brain micro motion is simulated, IL-36Ra levels were increased in astrocytes and microglia. In the same study, IL-36Ra levels were elevated after microwave electrodes were placed in adult mice [34]. In our study, IL-36Ra levels in Behcet’s patients with neurological involvement were higher than those without neurological involvement. This result is similar to the published literature.

IL-36α and IL-36γ levels were increased in inflammatory mucosal tissues of patients with inflammatory bowel disease [35]. A recent study identified that higher levels of IL-36α in fibrotic intestinal tissues from patients with Crohn’s disease and ulcerative colitis compared with control individuals [36]. In our study, no statistically significant difference was found in IL-36α and IL-36 Ra levels between patients with and without gastrointestinal involvement.

We believe the small sample size of our study was an important limitation and further studies are needed to elucidate the relationship between elevated IL-36Ra levels and activity of BS. Also we did not measure the level of IL-36Ra and IL-36α in long term follow-ups of BS patients. This makes us unable to determine the possible changes of serum levels of these cytokines in a particular BS patient over time regarding to the activity of the disease. IL-36 expression is enhanced upon response to a number of stimuli including cytokines, lipopolysaccharides, infections and smoke. We assessed the disease activity with presence of clinical findings and acute phase reactants, thus we did not use a standart Behcet’s syndrome activity scores because there is no gold standart test to evaluate disease activity and these scores had some limitations such as inconsistency between patients and physicians assessment, and
cultural differences [37]. Another limitation of our study is that this confounding factors was not evaluated [19]. Lastly, we performed our measurements on serum samples only. If obtained simultaneously, tissue samples from skin lesions or synovial fluids would have been valuable.

Conclusion

In conclusion, we found significantly higher serum levels of IL-36Ra in BS patients. Moreover this finding was associated with disease activity, serum CRP levels and clinical manifestations such as oral ulcers and neurological involvement. It is not clear whether such a slight difference has any clinical meaning, and should carefully consider the confounding factors which may affect the level of IL-36 α and IL-36Ra. This study supports that IL-36 cytokine family plays a role in the process of the disease, but further studies with larger groups are needed to confirm whether these cytokines can be used as disease-activity markers.

Conflict of Interest

The authors declare that they have no conflict of interest.

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