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Research

Serum Erythropoietin, Interleukin-4, Ferritine, Iron, Total Iron Binding Capacity, Vitamin B12, Folate Levels in Patients with Behçet's Disease

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Abstract

Background: Erythropoietin (EPO) is a naturally occurring hormone, produced in the kidneys, which stimulates the body to produce more red blood cells. Our purpose was to investigate EPO, IL-4 levels, and hematologic parameters including total blood count, iron, total iron binding capacity, ferritin, transferring, vitamin B-12, folate levels in patients with Behçet's disease (BD).

Material and Methods: Twenty patients with BD and 21 healthy volunteers were included in the study. Quantitative determination of EPO and IL-4 were determined with test kits by photometric ELISA method.

Results: We found a significant increase in EPO and folate levels, whereas serum iron levels decreased in patients with BD. The distribution of IL-4, total blood count, total iron binding capacity, ferritin, transferrine, vitamin B-12 levels were the same in both groups.

Conclusion: Patients with BD demonstrated higher EPO and folate levels, and lower serum iron levels. We conclude that higher EPO, folat levels may reflect a lower risk factor for systemic involvement of BD.

Introduction

Behçet's disease (BD) is a chronic, relapsing inflammatory disorder marked by eye inflammation, oral and genital ulcers, and certain other skin lesions, as well as varying, multisystem involvement including the joints, blood vessels, central nervous system, and gastrointestinal tract [1]. Although it has a worldwide distribution, BD is rare in the America and Europe and is more prevalent in Turkey and the Middle and Far East. It affects mainly young adults, with men having more severe disease than women [2].

The cause of BD is unknown. Genetic predisposition, autoimmune mechanisms, and viral infection are under consideration. Since vasculitis is the main pathologic lesion and circulating autoantibodies to human oral mucous membrane are found in approximately 50% of the cases, it is considered an autoimmune disease [3]. The diagnostic criteria established by the International Study Group for BD requires the presence of recurrent oral ulceration, in the absence of other clinical explanations, and two of the following: recurrent genital ulceration, eye lesions, skin lesions and/or a pathergy test [4]. This nonspecific skin inflammatory reacti-

vity denotes increased neutrophil chemotaxis and was once thought to be pathognomonic of BD. However, this reaction occurs in no more than 70% of the patients, usually in those with more extensive disease [1].

Erythropoietin (EPO) is a naturally occurring hormone, produced in kidneys, which stimulates the body to produce more red blood cells. Differentiation of elevated or depressed levels of EPO in serum can be helpful in assessing therapeutic options for the anemic patient and in the diagnostic evaluation of the patient with erythrocytosis [5]. It was indicated that higher EPO levels were significantly associated with higher levels of inflammatory markers such as C-reactive protein, interleukin [IL]-6, IL-1β, IL-1ra, IL-18, and IFN-γ [6]. In addition, circulating plasma concentrations of pro-inflammatory cytokines such as tumor necrosis factor [TNF]-α and IL -6 are elevated in patients with BD [7]. It was observed that IL-4 levels of were higher, lower or normal in patients with BD [8]. Hematologic abnormalities were observed in patients with recurrent oral ulceration [9]. Recently, Odabas et al. indicated increased serum ferritin levels in active BD [10].

There are no reports on EPO levels in patients with BD in the literature. Therefore we decided to investigate EPO, IL-4 levels, and hematologic parameters including total blood count, iron, total iron binding capacity (TIBC), ferritin, transferrine, vitamin B-12, folate levels in patients with BD.

Table 1. Biochemical Parameters in Patients

Parameters	Independent Samples t test	Patients (n=20) (mean±SD)	Control (n=21) (mean±SD)
IL-4	n.s	66,25 ± 19,62	53,87 ± 23,49
EPO	0,012	19,62 ± 15,79	9,73 ± 6,74
Kreatinin	n.s	0,73 ± ,17	0,65 ± 0,13
Urea	n.s	27,88 ± 5,75	25,89 ± 5,97
Iron	0,031	69,92 ± 33,22	96,73 ± 36,98
Iron BC	n.s	290 ± 82	287 ± 68
TIBC	n.s	363 ± 63	384 ± 53
Ferritine	n.s	52,46 ± 46,72	35,93 ± 33,69
Transferrine	n.s	3,09 ± 0,74	3,39 ± 0,56
B-12	n.s	364,5 ± 233,9	332,3 ± 113
Folate	0,025	10,71 ± 3,41	8,15 ± 2,93

Materials and Methods

Twenty patients (9 male-11 female) with BD were included in the study. Twenty-one healthy volunteers (8 male-13 female) constituted the control group. The diagnosis of BD was based on the International *Behçet*'s Study Group Criteria [4]. Control subjects were selected among healthy persons without a history of malignancy, atopy, renal disease, autoimmune and chronic diseases. The study was approved by the Ethics Committee of Mersin University.

Blood samples were collected in two different vacutainer tubes for measurement of EPO, IL-4 and routine biochemical analysis. Venous blood was sampled and tested for urea, creatinine, total blood count, iron, TIBC, ferritin, transferring, vitamin B-12, folate. For the EPO measurement samples were centrifugated at 700xg for 15 minutes. Then serum samples were stored at -70° C until EPO and IL-4 assayed. Quantitative determination of EPO (EPO ELISA; Roche Diagnostics GmbH, Mannheim, Germany) and IL-4 (h-Interleukin-4 ELISA; Roche Diagnostics GmbH, Mannheim, Germany) were determined with test kits by photometric Enzyme-Linked Immunosorbent Assay (ELISA) method. All samples were measured duplicate immediately. The concentrations of unknown samples were calculated in relation to the standard curve. Independent Samples t test was used for statistical analysis.

Results

The mean age of the patients and controls was 36,60±4,35 and 32,80±14,57 years respectively. There was no statistical significance between groups regarding their mean ages. Mean disease duration of patients was 9.05±10.35 years.

In the present study, as seen from the **Table-1**, we found a significant increase in EPO and folate levels whereas serum iron levels dec-

Table 2. Complete Blood Count Parameters

PARAMETERS	Patients (n=20) (mean±SD)	Control (n=21) (mean±SD)
WBC	7,54 ± 3,80	7,23 ± 2,31
RBC	4,62 ± 0,63	4,40 ± 0,51
HB	12,86 ± 1,41	13,11 ± 1,48
HTC	37,93 ± 3,77	38,10 ± 3,96
MCV	82,96 ± 8,97	86,75 ± 5,88
MCH	28,18 ± 3,73	29,35 ± 3,44
MCHC	33,91 ± 1,38	34,38 ± 1,41
PLT	230,6 ± 98,9	240,5 ± 84,9

reased in patients with BD. There were no statistical meaningfull differences between groups regarding to total blood count parameters. Results were given in **Table 2**.

In the correlation analysis, EPO levels showed statistically significant positive correlation with folate (r=0,374) levels. On the other hand EPO levels showed statistically significant negative correlation with TIBC (r=-0-,36) and transferrine (r=-0,36) levels.

Discussion

Erythropoietin, a 34 kDa glycoprotein hormone produced primarily by cells of the peritubular capillary endothelium of the kidney, is responsible for the regulation of red blood cell production. In premature as well as full term infants, the liver is the primary site of EPO production. The kidney becomes the primary site of EPO synthesis shortly after birth. EPO is the only haemapoietic growth factor whose production is regulated by hypoxia. EPO stimulation was essential for the survival and proliferation of colon-forming units-erytroid. Several studies showed that the number of EPO receptors found at the cell surface of erythroid cells. Circulating EPO binds to EPO receptors on the surface of erythroid progenitors resulting in replication and maturation to functional erythrocytes by an incompletely understood mechanism. EPO receptors were also detected in megakaryocytic cells, endothelial cells and in cells with neuronal characteristics [**5**].

Clinical conditions that give rise to tissue hypoxia including anemia, lung disease, or cyanotic heart disease, lead to increased levels of serum EPO. In anemia, serum EPO levels do not rise above normal until hemoglobin levels fall below 110 g/L. As may be expected in patients with renal insufficiency, serum EPO levels remain inappropriately low despite the anemia. However, inappropriately low serum EPO levels may also be seen in anemic patients with cancer, as well as those with rheumatoid arthritis, HIV infection, ulcerative colitis, sickle cell anemia, and the anemia of prematurity. The mechanism of the inappropriate EPO response varies. For example, a primary production defect is apparent in renal disease and the anemia of prematurity; suppression of EPO synthesis by inflammatory cytokines (e.g., IL-1, TNF-"alpha") is believed to occur in certain chronic diseases or cancer [**5, 6**].

Patients with chronic renal failure have activation of various immune cells, both monocytes and T-cells. These mononuclear cells have also been shown to release pro-inflammatory cytokines such as IL-1, IL-6, TNF-α and interferon gamma. These cytokines, particularly TNF-α and interferon gamma, are known to cause significant suppression of erythropoiesis. The exact molecular mechanism for this effect is not yet clear, but interferon gamma is an important stimulator of apoptosis in various cell types, including erythroid progenitor cells. This effect may be potentiated by other cytokines such as TNF-α, and this might then antagonise the anti-apoptotic action of erythropoietin on erythroid progenitors cells, thus reducing responsiveness to exogenous erythropoietic therapy.

Differentiation of elevated or depressed levels of erythropoietin in serum can be helpful in assessing therapeutic options for the anemic patient and in the diagnostic evaluation of the patient with erythrocytosis [**5**]. Recently, Ferrucci et al indicated that higher EPO levels were significantly associated with higher levels of inflammatory markers such as C-reactive protein, IL-6, IL-1β, IL-1ra, IL-18, and IFN-γ [**6**]. Circulating plasma concentrations of pro-inflammatory cytokines (e.g. TNF-α and IL-6) are elevated in patients with BD7. We found that EPO levels were higher in patients with BD. High levels of EPO may lead to imbalance of inflammatory cytokine. Pro-inflammatory cytokines might be alter due to EPO activity in patients with BD. Gibson et al report a case of thrombotic leg ulceration associated with recombinant human EPO use [**11**]. An activated hemostatic system with venous and arterial thrombosis and an elevated compensatory fibrinolytic process are among the clinical features of BD [**12**]. EPO is a novel cytoprotective agent in both neuronal and vascular systems [**13**]. Our patients have no vascular and neurological involvement. All patients have only mucocutaneous involvement.

In BD, the result regarding Th2-phenotype lymphocytes and cytokines are controversial. Some studies have shown decreased levels CD8+ T lymphocytes, IL-4 and IL-10 [**14, 15**], whereas some others demonstrated increased CD8+ T lymphocytes as well as increased

serum concentrations of IL-4, IL-6, IL-10 and IL-13, indicating a reduced circulating CD4+/CD8+ ratio [**16, 17**]. In our study, the levels of IL-4 were found to be high in our patients with BD. But it wasn't statistically significant. It may due to our small patient group.

Hematologic abnormalities were observed in recurrent oral ulceration (ROU) [**9**]. *Odabas* et al. indicated increased serum ferritin levels in active BD. They concluded serum ferritin levels do not reflect serum iron levels [**10**]. *Challacombe* et al. noted that the mean ferritin concentrations in patients with BD, ROU, or withother oral ulcers were significantly reduced in comparison with controls [**9**]. Erythrocyte selenium, plasma iron, manganese, and zinc levels are decreased, whereas plasma copper, erythrocyte zinc and manganes levels are elevated in patients with BD [18]. In our study, we found higher folate levels and lower serum iron levels. The result regarding folate levels are controversial in patients with BD. *Korkmaz* et al. observed normal folate levels [**19**]. Whereas *Yesilova* et al. found lower folat levels in patients with BD [**20**]. It has been demonstrated higher plasma homocystein concentrations and lower vitamin B12 and folate levels in BD patients with thrombosis and ocular involvement than those without [**20**]. We believe that the low frequency of vascular and ocular involvement of our patients may be result of the beneficial effect of high folat levels. We found lower serum iron levels in our study. It might reflect chronic disease. The other hematologic parameters including serum ferritine, total iron binding capacity, vitamin B12 levels were normal in our series.

In our patient group, high levels of EPO and folate, and low levels serum iron concentrations were found. However we did not detect any relationship between BD and serum ferritine, TIBC, vitamin B12 levels. We conclude that higher EPO levels may reflect a lower risk factor for systemic involvement of BD. EPO may have a role in modulating endothelial function and may be involved in mechanisms for vessel endothelium repair during an exacerbation of BD. *Keast* el al. have indicated the treatment of chronic skin ulcers in individuals with anemia of chronic disease using recombinant human EPO [**21**]. Human recombinat EPO might show promise in resolving the systemic involvement of BD. Further studies in larger patient series are needed to determine the prevalence and distribution of EPO in patients with BD and whether deficiency of EPO constitute a major risk factor in the development of systemic manifestations of BD.

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