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Serum osteoprotegerin (OPG) measurement in Behcet's disease

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Aim: Recent studies indicate that osteoprotegerin (OPG) acts as an important molecule in the development of vascular diseases. The aim of the present study was to examine the association between serum OPG levels and Behcet's disease (BD).

Materials and methods: Twenty-five patients with BD and 20 healthy control subjects, matched for age and sex, were included in this study. Serum levels of OPG were determined using ELISA.

Results: We found that serum levels of OPG were significantly higher in patients with BD than in healthy control subjects (813.75 ± 11 vs. 655.56 ± 17 pg/mL, P = 0.002). There was no difference between active patients and those in remission.

Conclusion: Collectively, serum OPG levels were increased in BD patients, suggesting that OPG might be linked with the development of vasculitis or other inflammatory processes. It was determined that OPG cannot be used as a monitoring test by itself; however, it might be a useful marker for supporting clinical and laboratory findings in relation with BD. Further detailed studies are necessary for a more definitive evaluation of these results.

Key words: Behcet's disease, osteoprotegerin

Behçet hastalarında serum osteoprotegerin ölçümü

Amaç: Son çalışmalar osteoprotegerinin (OPG) özellikle vasküler hastalıkların gelişiminde önemli bir molekül olduğunu göstermektedir. Burada sunulan çalışmanın amacı, Behçet hastalığı ile serum osteoprotegerin düzeyleri arasında bir ilişki olup olmadığını araştırmaktır.

Yöntem ve gereç: 25 Behçet hastası ile cins ve yaş olarak uyumlu 20 sağlıklı kontrol çalışmaya dahil edildi. Serum OPG düzeyleri ELISA ile değerlendirildi.

Bulgular: Behçetli hastalardaki serum OPG düzeyleri (813,75 ± 11 pg/mL) sağlıklı kontrollerden (655,56 ± 17 pg/mL) anlamlı olarak yüksek bulundu (P = 0,002). Ancak, aktif dönemdeki hastalarla remisyondakiler arasında OPG düzeyleri yönünden anlamlı bir fark yoktu.

Sonuç: Behçet hastalarında OPG değerlerinin yüksek bulunması, OPG'nin vaskülitin veya diğer inflamatuar olayların gelişimiyle ilgili olabileceğini göstermektedir. Sonuç olarak, OPG tek başına hasta izleminde kullanılabilecek bir test olarak değerlendilmese de Behçet hastalarında klinik ve laboratuar bulguları desteklemek için yararlı bir gösterge olabilir. Bu sonuçları daha iyi değerlendirmek için detaylı çalışmalara ihtiyaç vardır.

Anahtar sözcükler: Behçet hastalığı, osteoprotegerin

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Introduction

Osteoprotegerin (OPG) and its ligand (RANKL: Receptor activator of nuclear factor kappa-light-chain-enhancer of activated B-cells) have key roles in controlling the orderly development and function of the immune system. OPG ligand is abundantly produced by activated T lymphocytes. It prevents apoptosis and prolongs survival of dendritic cells, which also express large amounts of the receptor for OPG ligand (1). Activated T cells are an important source of OPG ligand, and dendritic cells are specific targets of the ligand (2). Behcet's disease (BD) is an inflammatory vascular disease. Since vascular diseases are promoted by immune-mediated mechanisms, it was hypothesized that OPG ligand, its antagonist, and OPG, may also form an important cytokine system in vascular biology. OPG is an autocrine developmental factor in endothelial cells (3). Some researchers have found abundant expression of OPG in the media of the great arteries and in-vitro analyses revealed OPG production by arterial smooth-muscle cells and endothelial cells. These producing areas support its role on the vascular area. Osteoprotegerin mRNA and protein levels were very low in endothelial cells plated on the non-integrin cell attachment; however, osteoprotegerin mRNA and protein levels were induced 5–7-fold following αvβ3 ligation by osteopontin. Interestingly, OPG was upregulated by the binding of $\alpha_v\beta_3$ integrin (vitronectin) with osteopontin and was found to represent an anti-apoptotic signal for endothelial cells, which suggested an important role for OPG in maintaining the integrity of the luminal surface of the vascular wall (4). OPG synthesis was increased with some cytokines such as TGF-β, TNF-α, IL-1 α, and IL-18. Some of these cytokines have a proinflammatory role (IL-1α, TNF-α,) and stimulatory effect on T cells, especially Th1 cells (IL-18). BD is strictly related to T cell stimulation and inflammation; in this respect, OPG can stimulate inflammatory and cell mediated immune response related to cytokines. Recent studies indicate that OPG acts as an important molecule in the development of different vascular diseases. The aim of the present study was to examine the association between serum OPG levels and BD.

Materials and methods

This study was approved by the Local Ethical Committee of Fırat University, Elazığ, Turkey. Patients were diagnosed with BD according to the diagnostic criteria of the International Study Group for BD. Twenty-five BD patients and 20 age- and sex-matched healthy control subjects were included in this study. Thirteen patients were in active periods and the remaining 12 patients were in inactive periods. At the time of the clinical assessment, patients were included in the active group if they had at least 2 of the following clinical findings: oral ulcers, genital ulceration, active uveitis, recent arthritis, papulopustular or pseudofollicular cutaneous lesions, neurological involvement, and pathergy test positivity. Activity criteria: Totally, 18 BD patients had ocular complications (6 with active ocular attacks, 4 with inflammatory arthritis, and the rest uveitis with remission period), 7 patients had mucocutaneous lesions (3 were in the active period and the rest in remission).

Peripheral venous blood samples (2 mL) were collected in biochemical tubes. Quantitative measurements of human OPG levels in the serum were made using RayBiotech, Inc. Human OPG ELISA kits, according to the manufacturer's protocol, on a Dynex 1DXC-1381 (USA) fully automatic ELISA system. This assay is a sandwich ELISA, using a biotinylated antihuman OPG for detection. The plates were read at 450 nm. The minimum detectable dose of OPG was less than 1 pg/mL.

Statistical analyses were carried using SPSS 11.0 for Windows (SPSS, Inc., Chicago, IL, USA). The Mann-Whitney U and Kruskal-Wallis tests were used and a P value of less than 0.05 was considered statistically significant.

Results

The mean age of the total BD patients (14 females and 11 males) was 44 years (range: 20-57 years) and the mean duration of disease was 6.16 years (range: 1-14 years). Baseline characteristics and the therapeutic drugs used by patients with BD are presented in the Table.

Table. Baseline characteristics of patients with BD.

Patient characteristics	Inactive (n: 13)	Active (n: 12)
Age (year)	36.15	**42.25**
Duration of disease (year)	7.30	**9.58**
Eye involvement (%)	69.23	**41.66**
Mucocutaneous lesion (%)	11.38	**33.33**
Arthritis and musculoskeletal involvement (%)	15.38	**25.00**
Sumatriptan use (%)	30.76	**33.33**
Sulfasalazine use (%)	30.76	**16.66**
Colchicine use (%)	61.53	**75.00**
Prednisolone use (%)	**15.38**	**25.00**

We found that the serum levels of OPG were significantly higher in patients with BD compared to the healthy control subjects (813.75 ± 11 vs 655.56 ± 17 pg/mL, P = 0.002). There was no difference between the active patients and those in remission (Figure).

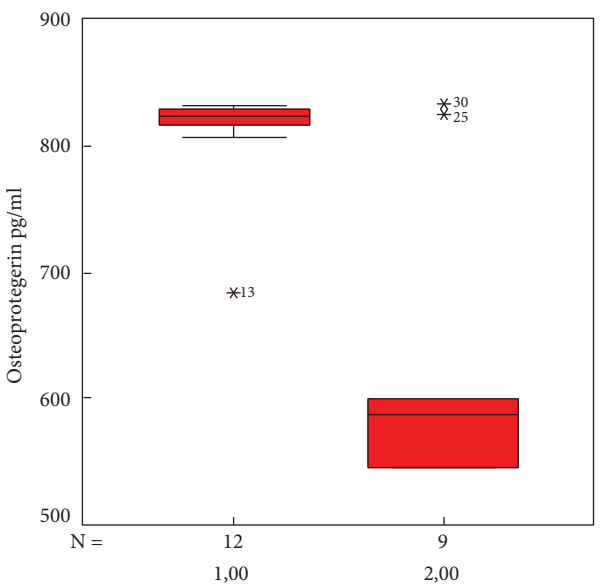

Figure. The distribution of osteoprotegerin levels in the patient and control groups.

Discussion

Recent studies indicate that OPG acts as an important molecule in the development of vascular diseases. The major aim of this study was to determine whether serum measurement of OPG, which can be easily performed in daily clinical practice, has a diagnostic or monitoring value in BD patients. The other aim of the present study was to examine the association between serum OPG levels and active or inactive periods of BD patients.

We found that serum levels of OPG were significantly higher in the patient's with BD compared to healthy control subjects (P = 0.002). However, the levels measured in some healthy controls were similar to those found in BD patients. Based on our results, we conclude that the assessment of OPG serum levels in BD may be of interest from a pathophysiological point of view; however, its clinical role as a routine biochemical marker remains questionable.

In one study, it was revealed that OPG levels are associated with markers of systemic inflammation in patients with psoriatic arthritis (5). In another study, 40 patients with systemic lupus erythematosus (SLE) were evaluated, of whom 20 had active disease. Osteoprotegerin may have an additional function due to its ability to bind and inhibit the members of the tumor necrosis factor (TNF)-superfamily, such

as TNF-alpha and TNF-related apoptosis inducing ligand (TRAIL). Mean serum TRAIL concentrations were elevated in SLE patients, and were higher than in the healthy control subjects or in other patients with immune-mediated diseases (rheumatoid arthritis or Wegener's granulomatosis) (6). High OPG levels might be the result of compensatory production during acute and subacute phases of Kawasaki disease. Additionally, OPG assay might be a clinically useful marker to monitor and differentiate patients who develop such coronary artery abnormalities from those who do not develop them (7). In another study, serum OPG levels were increased in SLE patients with antiphospholipid syndrome (APS) and correlated with titers of antiphospholipid antibodies, suggesting that OPG might be linked to the development of APS (8). There are, however, a number of physiological effects of OPG that were not discussed and which demonstrate the depth of influence of the RANK/RANKL/OPG system on both inflammatory disease and, possibly, immune surveillance mechanisms. OPG has a role in mucosal inflammation and it can be related to mucosal inflammation and oral aft in BD (9). Recently, the OPG/RANKL/RANK axis has been implicated in various inflammatory responses and has also been linked to atherogenesis and peripheral vascular disease (10). It might be suggested that OPG may not only have a prominent role in the vascular or mucosal inflammation associated with BD, but it could also have considerable significance in the abnormal immune activation characterizing BD. Alternatively, elevated OPG in BD may reflect T-cell activation; however, further functional studies are necessary to clarify its respective role in BD. We concluded that OPG levels not only reflect vascular involvement but also affected uncontrolled systemic inflammatory situation like other diseases with or without vasculitis.

Currently, the measurement of OPG levels alone does not constitute a definitive characteristic test; however, it might be a useful marker for supporting clinical and laboratory findings in monitoring BD. Additional detailed studies are necessary for further evaluation of these results.

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