# Oxidant/Antioxidant Parameters And Their Relationship With Behcet's Disease

## Oksidan/Antioksidan Parametrelerin Behçet Hastalığı ile İlişkisi

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**Abstract**

**Purpose:**Behcet's disease is a chronic inflammatory disorder of unknown aetiology. It has been postulated that an imbalance of the oxidant and antioxidant systems related to the disease are important in its pathogenesis. We aimed, for the first time, to evaluate the xanthine oxidase, paraoxonase1 activities and thiol levels altogether in patients with Behcet's disease in the active stage of the disease.

**Material and Methods:**Serum xanthine oxidase, paraoxonase1 activities and thiol levels were measured spectrophotometrically in 14 patients with Behcet's disease and in 15 healthy subjects who constituted the control group.

**Results:**The serum xanthine oxidase activity was statistically significant and higher in the patient group than controls. paraoxonase1 activity and thiol levels were found to be lower in the Behcet's disease group than the control group.

**Conclusion:**Increase in reactive oxygen species in the active stage of the disease in Behcet's disease may results in a pro-oxidation environment which in turn results in increased xanthine oxidase activity and decrease in paraoxonase1 activity and thiol levels.

Key Words: **Behcet Syndrome; PON1 protein; Sulfhydryl Compounds; Xanthine oxidase.**

**Özet**

**Amaç:** Behçet hastalığı, etyolojisi bilinmeyen kronik enflamatuar bir hastalıktır. Hastalığın patogenezinde, oksidan ve antioksidan sistemler arasındaki dengesizliğin önemli bir faktör olduğu varsayılmaktadır. Behçetli hastalarda ilk defa, ksantin oksidaz, paraoksonaz1 aktivitelerini ve tiyol seviyelerini birlikte değerlendirmeyi amaçladık.

**Gereç ve Yöntemler:**Hastalığının aktif döneminde bulunan 14 Behçet hastasında ve 15 sağlıklı kişiden oluşan kontrol grubunda, serum ksantin oksidaz, paraoksonaz1 aktiviteleri ve tiyol seviyeleri spektrofotometrik yöntemlerle ölçüldü.

**Bulgular:**Serum ksantin oksidaz aktivitesi hasta grubunda, kontrol grubuna göre istatistiksel olarak anlamlı yüksek bulundu. Bununla birlikte, paraoksonaz1 aktivitesi hasta grubunda, kontrol grubuna göre daha düşük bulundu. Ek olarak tiyol seviyeleri hasta grubunda, kontrol grubuna göre daha düşük bulundu. Ayrıca, serum tiyol düzeyleri, ksantin oksidaz aktivitesi ile negatif, paraoksonaz1aktivitesi ile pozitif korele bulundu.

**Sonuç:**Behçet hastalığının aktif döneminde reaktif oksijen türlerindeki artış, ksantin oksidaz aktivitesinde artma, paraoksonaz1 aktivitesi ve tiyol düzeylerinde azalma ile sonuçlanan prooksidan şartların oluşmasına neden olabilir. Bu sonuçlara dayanarak, ksantin oksidaz aktivitesini inhibe eden ve paraoksonaz1 aktivitesini yükselten antioksidan ajanlarla yapılacak effektif bir antioksidan terapi, Behçet hastalarında tedavi seçeneği olabilir.

Anahtar Kelimeler: **Behçet hastalığı; Ksantin oksidaz, PON1 Protein ;Sülfidril bileşikler.**

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## Introduction

Behçet's disease (BD) was first described by Hulusi Behçet, a Turkish dermatologist, in 1937 as a triad of relapsing iridocyclitis with oral and genital ulcers (1). It is now recognized as a polysymptomatic systemic vasculitis which may present with skin lesions, arthritis, central nervous system manifestations and vascular involvement in addition to the original triad. Ocular manifestation has been an indicator of severe prognosis and 15-25% of affected patients undergo blindness (2, 3).

The etiology and pathogenesis of BD have not been clarifed. There is increasing evidence indicates that oxidative stress is increased in BD, owing to overproduction of reactive oxygen species (ROS) and decreased efficiency of antioxidant defences (4, 5).

It has been suggested that source of increased ROS is activated neutrophils which lead to tissue injury in BD (6). Another source of ROS, There is growing evidence is that, superoxide radicals generated by xanthine oxidase (XO), which are primarily responsible for the cellular deterioration associated with several conditions (7, 8). XO catalyzes the conversion reactions of hypoxanthine to xanthine to uric acid, the last reactions in the purine metabolism, with by product of toxic superoxide radical. In this regard, it is a key enzyme between purine and free radical metabolism and hence the increased XO activity may cause further tissue damage because of its free radical-generating effect (9). Xanthine dehydrogenase/oxidase is present as two isoforms in vivo. In most tissues the NAD+-dependent dehydogenase predominates but when oxidation of its thiol groups, or when it undergoes limited proteolysis, it is converted to its oxidase form. Uric asid production is coupled with the formation of reactive oxygen species when the enzyme is in its oxidase form (10, 11).

Blood paraoxonase (PON1) is a calcium-dependent esterase that is known to catalyze hydrolysis of organophosphates, and is widely distributed among tissues such as liver, kidney, intestine, and also plasma (12, 13). PON1, which is exclusively bound to high-density lipoprotein (HDL), is recognized as an antioxidant enzyme, because it hydrolyses lipid peroxides in oxidized lipoproteins (14).

It is reported that the protection against lipid peroxidation is accomplished by PON1; during this procedure, free sulfhydryl groups of PON1 interacts with specific oxidized lipids and in doing so, PON1 is consequently inactivated (15).

Thiol groups are important members of the antioxidant team as they have been shown to destroy ROS and other free radicals by enzymatic as well as non-enzymatic mechanisms (16).

This is the first study, which has been performed to evaluate the oxidative stress hypothesis by measuring the XO activity, PON1 activity and thiol levels altogether in patients with BD.

## Materials and Methods

**Patients.** In this study, we measured serum XO activity, PON1 activity and thiol levels in 14 patients with BD who were followed up by Uvea-Behcet clinic of the Ophthalmology Department of Erciyes University Medical Faculty, Kayseri, Turkey and who were hospitalized in the eye clinic because of uveitis in the active stage of the disease. Results are compared with age and sex matched 15 healthy controls. Diagnosis of BD was made according to the criteria set by the International Study Group for Behcet's Disease which necessitates the presence of oral ulceration plus any two of genital ulceration, typical defined eye lesions, typical defined skin lesions, or a positive pathergy test (17). In all patients during the active stage, oral, genital and eye lesions were present and in the majority, skin lesions were also present. Systemic evaluation of the patients and the controls was made by an internal medicine specialist.

**Chemicals.** All chemicals used in this study were from Sigma Chemical Co. (St. Louis, MO, USA) and were of analytical grade or the highest grade available.

**Samples.** Informed consent was obtained from patients prior to the study. The study protocol and the procedures were approved by Erciyes University Ethical committee and were in accordance with the Helsinki Declaration of 1975.

All blood samples were collected in the morning after an overnight fast, and serum samples stored at - 70 C until assay for XO, PON1 activities and thiol levels.

**Determination of Xanthine oxidase activity.** Serum XO activity was measured by the method of Prajda and Weber, where the activity is measured by determination of uric

acid from xanthine. Plasma (50 L) was incubated for 30 min at 37 0C in 3 mL of phosphate buffer (pH 7.5, 50 mM) containing xanthine (4mM) (18). The reaction was stopped by addition of 0.1 mL 100% (w/v) TCA, the mixture was then centrifuged at 4000 g for 20 min. Urate was determined in the supernatant by measuring absorbance at 292 nm against a blank and expressed as units per milliliter (U/mL) in serum.

**Determination of PON1 activity.** Serum PON1 activity was measured according to a method described elsewhere (19). We measured the rate of hydrolysis of paraoxon by monitoring the increase of absorbance at 405 nm and at 25 ℃. The basal assay mixture included 1.0 mM paraoxon and 1.0 mM $CaCl_2$ in 0.05 M glycine buffer pH 10.5. One unit (IU) of paraoxonase activity is defined as 1 mol of p-nitrophenol formed per min, and activity was expressed as U/L of serum.

**Determination of thiol levels.** A spectrophotometric assay based on 2,2-dithiobisnitrobenzoic acide (DTNB or Elman's reagent) is used for thiol assay (20). An aliquot of serum is mixed with Tris-EDTA buffer, than DTNB is added. After 15 minute incubation at room temperature, the absorbance is measured at 405 nm. A reagent blank without sample and a sample blank with methanol instead of DTNB were prepared in a similar manner. GSH (50-100 mol/L) solution is used as calibrator. Thiol levels were expressed as imol/L.

**Statistical analysis.** Statistical evaluation was carried out with the SPSS 11.0 (Statistical Packages for Social Sciences; SPSS Inc, Chicago, Illinois, USA). Data obtained from the study groups were compared by the non-parametric Mann Whitney U test; correlation analyses between variables were made by Spearman' test; p value less than 0.05 was considered as statistically significant. All the results were expressed as "mean with their standard deviation" (mean ± SD).

## Results

There was no statistically significant difference of age and sex distribution between patients with BD (6 men, 8 women mean age ± SD: 37.0±9.8 years) and control (5 men, 8 women mean age±SD: 35.1±9.7 years) groups (p0.05).

The serum XO activity was higher in the patient group (1.6± 0.2 U/mL) than controls (1.1±0.4 U/mL; p<0.003). PON1 activity was found to be lower in the BD group (159.7±52.3 U/L) than the control group (248.1±95.0 U/L; p<0.008). Thiol levels was found to be lower in the BD group (238.3±78.9 μmol/L) than the control group (292.0±44.5 μmol/L; p<0.038).

Serum thiol levels were found to be negatively correlated with XO activity (r=-0.384, p=0.048), positively correlated with PON1 activity (r=0.404, p=0.037). The results are presented in table I and II.

## Discussion

The etiology and pathogenesis of Behçet's disease are unknown. ROS generation has been proposed as factors in the etiology and pathophysiology of BD (21). It has been reported that the vascular and endothelial tissue damage in BD may reflect enhanced production of ROS (22).

We found increased XO activity in patients with Behcet's disease. The increased XO activity is an indicator of free radical production in BD. There is a similar study indicating that the increasing level of XO activity in also BD (23)

In sera or plasma of healthy people, XO activity is normally absent or only present at very low levels (24) but there is growing evidence that superoxide radicals generated by XO are primarily responsible for the cellular deterioration associated with several conditions. It increases markedly in various liver disorders (25), bladder cancers (7) and preeclampsia (8). We clearly suggested that the oxidative stress might be due to increased XO activity in BD. XO exists in oxidase and dehydogenase isoforms. In most tissues the NAD-dependent dehydogenase predominantes, but when oxidation of its thiol groups occur, or when it undergoes limited proteolysis (10, 11). The enzyme exists primarily as the xanthine dehydogenase form and can be converted to XO by a variety of conditions including proteolysis, homogenization, sulfhdryl oxidation, storage at –20℃ and anaaerobiosis (26).

It has been previously reported that adenosine deaminase activity was increased in BD providing some evidences for a potential role of T lymphocyte activation (23). In BD, increased T cell activation leads to an excess of purines with increased availability of substrate to NAD-dependent dehydogenase/XO. It has been also proposed that process of increased substrat formation could be an important factor in trigering XO-mediating free radical

**Table I.** XO, PON1 activities and thiol levels in Behcet's Disease (BD).

| Parameters | BD | Control | p |
|---|---|---|---|
| XO Activity (U/mL) | 1.6±0.2 | 1.1±0.4 | *0.003 |
| PON1 Activity (U/L) | 159.7±52.3 | 248.1±95.0 | *0.008 |
| Thiol (μmol/L) | 238.3±78.9 | 292.0±44.5 | *0.038 |

*The serum Xanthine Oxidase (XO) activity was higher in the patient group than controls. Blood Paraoxonase (PON1) activity was found to be lower in the BD group than the control group. Thiol levels was found to be lower in the BD group than the control group.

generation (10). In our opinion, the possible reason of increased XO activity in BD may be depend on T cell hyperfunction.

In the ischemia/reperfusion studies, it was demonstrated that ischemia results in the accumulation of purines (hypoxanthine and xanthine) from catabolism of ATP, and from xanthine dehydrogenase to XO conversion (10, 11). This conversion from xanthine dehydogenase to XO has not been yet investigated in BD. In our study, increased XO activity in BD may be due to increased T cell activation and increased degradation of ATP.

It has been suggested that activated leukocytes produce cytokines that irreversibly convert endothelial xanthine dehydogenase to xanthine oxidase causing endothelial release of ROS (8). Above mentioned mechanism could be accounted as another possible mechanism. Plasma XO is localized on endothelial cell surface membranes (27) and because the endothelium has a very important role in regulating vascular tone, excessive production of reactive oxygen species in the endothelium may lead to endothelial dysfunction (28). Endothelial dysfunctions, which give rise to reactive oxygen species arising from increased XO activity. The most common factors affecting the initiation and development of atherosclerosis are endothelial cell damage and oxidized lipoproteins, especially low density lipoprotein (22). Endothelial damage and increased XO activity in BD may results in a pro-oxidation environment in the subendothelial region. Thus, the process which may be a cause of atherosclerosis may be initiated by the oxidation of LDL. PON1 is known to be an important enzyme in this process. There are evidence that decreasing PON1 activity is also play a role in the atherosclerosis (14, 15) PON1 is known for, probably via production of super oxide anions produced by XO, inactivated by ROS (29). Consumption of PON1 for prevention of oxidation usually leads to variation in serum PON1 activity. In addition, we have previously shown that PON1 activity was decreased in some diseases due to ROS pathogenesis under oxidative stress and inflammation condition (30-33).

It is reported that the protection against lipid peroxidation is accomplished by PON1; during this procedure, free sulfhydryl groups of PON1 interacts with specific oxidized lipids and in doing so, PON1 is consequently inactivated (29).

**Table II.** Correlation with parameters in BD.

| Parameters | r | p |
|---|---|---|
| *Thiol-PON1 | 0.404 | 0.037 |
| *Thiol-XO | -0.384 | 0.048 |

*Serum thiol levels were found to be negatively correlated with XO activity, positively correlated with PON1 activity.

Therefore, it can be speculated that superoxide anions could be one of the other responsible factors for decreased PON1 activation, via changing the protein structure. Another possible mechanism related to the decreased PON1 activity may result from a suppression of PON1 synthesis accordingly due to genetic defect. At the present study, thiol levels were found to be lower. There are similar studies in accordance with our present study (34). It has been also known that thiol groups are main antioxidants agents (16). This could be appropiated as an indicator of oxidative stress.

There was a negative correlation found between thiol levels and XO activity and in addition a clear positive correlation was observed between thiol levels and PON1 activity. Thiol is known as an important antioxidant enzyme, therefore the decrease in its level may cause also increase in XO level which is known to be as an oxidant agent, incontrast to that it causes a decrease in PON activity which is known to be antioxidant enzyme. The common features for these two enzymes is that they both become inactivated by oxidation of thiol groups. These findings support our hypothesis.

This study is the first one that thiol, XO and PON1 activities assessed all in one and the possible existancy of correlations among them has also been shown altogether in BD. Since total thiol is also an antioxidant agent, effective antioxidant therapy to inhibit XO activity is necessary and agents to increase PON1 activity may be a therapeutic option in BD. In conclusion, increase in ROS in the active stage of the disease in BD may results in a pro-oxidation environment which in turn results in increased XO activity and decrease in PON1 activity and thiol levels.

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