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THE EFFECTS OF COLCHICINE AND VITAMIN E ON THE LIPID PEROXIDATION AND ANTIOXIDANT ENZYMES IN PATIENTS WITH BEHÇET'S DISEASE

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ABSTRACT

Purpose: The etiopathogenesis of Behçet's disease (BD) remains unknown, while increasing attention has been focused on the role of excessive production of free radicals produced by activated neutrophils. The purpose of the study was to assess the oxidant/antioxidant status in BD and to evaluate the effects of colchicine and vitamin E therapies on these systems.

Materials and Methods: The levels of plasma malondialdehyde (MDA), and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were measured in 20 patients in the active stage of the disease and in 20 healthy controls. These measurements were repeated in patients with BD after three months of therapy with either colchicine 1.5 mg/day or colchicine plus 400 IU vitamin E.

Results: Higher MDA levels and lower SOD and GSH-Px activities were observed in the patients when compared with the controls ($p<0.05$). After three months, MDA levels decreased, and SOD and GSH-Px levels tended to increase in the colchicine and colchicine plus vitamin E groups compared to the pretreatment levels ($p<0.05$). No statistically significant difference was observed between the treatment groups.

Conclusion: Our results showed that lipid peroxidation is increased and erythrocyte antioxidant capacity is decreased in patients with active BD, and that colchicine alone and colchicine plus vitamin E have beneficial effects on antioxidant defense systems in BD.

Key Words: Antioxidant Enzymes, Behçet's Disease, Colchicine, Lipid Peroxidation, Vitamin E.

BEHÇET HASTALARINDA SİSTEMİK KOLŞISIN VE KOLŞİSİN +VITAMIN E'NİN LİPİD PEROKSIDASYONU VE ANTİOKSİDAN ENZİMLERİ ÜZERİNE ETKİLERİ

ÖZ

Amaç: Behçet hastalığının etyopatogenezi bilinmemektedir. Bununla birlikte aktif nötrofillerin artmış serbest radikal üretimleri üzerinde durulmaktadır. Bu çalışmanın amacı Behçet hastalrında oksidan/antioksidan durumun değerlendirilmesi ve kolşisin ve 400 IU E vitaminin bu sistemler üzerine etkisinin değerlendirilmesidir.

Gereç ve yöntemler: Bu amaçla aktif dönemdeki 20 Behçet hastası ve 20 sağlıklı kontrolde plazma malondialdehit (MDA), süperoksid dismütaz (SOD) ve glutatyon peroksidaz (GSH-Px) seviyeleri ölçülmüştür. Behçet hastalarında bu değerler üç ay süreyle kolşisin veya kolşisin + E vitamini tedavisini takiben tekrar ölçülmüştür.

Bulgular: Kontrol grubu ile karşılaştırıldığında aktif dönem Behçet hastalarında MDA seviyeleri yüksek, SOD ve GSH-Px aktiviteleri ise daha düşük olarak tespit edilmiştir. ($p<0.05$). Üç aylık tedaviler sonunda Kolşisin ve kolşisin + E vitamini gruplarında tedavi öncesi değerleri ile kıyaslandığında MDA seviyelerinde azalma, SOD ve GSH-Px seviylerinde ise artma tespit edilmiştir ($p<0.05$). Tedavi grupları arasında istatistiksel olarak anlamlı bir fark gözlenmemiştir.

Sonuçlar: Sonuçlarımız aktif Behçet hastalarında lipid peroksidasyonunun arttığı ve eritrosit antioksidan kapasitenin azaldığını ve Kolşisin ve E vitaminin Behçet hastalığında antioksidan sistemler üzerine faydalı etkileri olduğunu göstermektedir.

Anahtar Kelimeler: Antioksidan Enzimler, Behçet Hastalığı, Kolşisin, Lipid Peroksidasyonu, Vitamin E.

INTRODUCTION

Behçet's disease (BD) is a multisystem disorder characterized by chronic inflammation.[1] The aetiology of the disease remains unknown, while many studies have demonstrated an increase in various functions of leukocytes in peripheral blood such as chemotaxis, phagocytosis, and superoxide radical anion generation. Neutrophil hyperactivity and free radicals are thought to be responsible for oxidative tissue damage seen in BD.[2-7]

Colchicine is widely used in the treatment of BD in Turkey. It is effective in controlling the mucocutaneous symptoms of the disease. Studies have shown high concentrations of colchicine in leukocytes and inhibitory effects on some leukocyte functions, e.g., adhesiveness, mobilization, and degranulation of lysosomes.[8,9]

Reactive oxygen species (ROS) have a critical role in the immune system and the process of inflammation.[10,11] Many studies have demonstrated abnormalities in the oxidant and antioxidant systems in BD.[11-14] ROS produced in excessive amounts may cause toxic effects described as oxidative damage on tissues.[12,15-19] The oxidation of membrane lipids is one of the primary events in oxidative cellular damage.[20] Lipid peroxides are generated by free radical chain reactions, which can be initiated by oxygen radicals that are increased in surrounding tissue. To determine the degree of lipid peroxidation induced by ROS, levels of breakdown products such as malondialdehyde (MDA) are measured.[13,21,22] Many studies have demonstrated that lipid peroxidation is increased in patients with BD.[13,16,17,23-25]

As erythrocytes are prone to peroxidation, the levels of antioxidants in erythrocytes from active BD patients are good indicators for the oxidative stress found in the disease. The antioxidant capacity in erythrocytes mainly consists of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). SOD constitutes the first defense mechanism against oxidative damage and GSH-Px plays a role in the reduction of peroxides to nontoxic alcohols.[11,22] Serum SOD levels are elevated in a wide variety of inflammatory diseases. SOD levels have been studied in patients with BD but the results are contradictory.[12,14,26,27]

Erythrocytes are protected against oxidative stress mainly by GSH-Px.[28,29] The decrease in the activity of GSH-Px causes the accumulation of hydrogen peroxide, which is very toxic to cells.[22]

Vitamin E is a mixture of compounds called tocopherols.[10,22] Vitamin E is highly lipid soluble and is mainly distributed in membranes and lipoproteins, where it acts at multiple sites to break free-radical chain reactions such as lipid peroxidation.[20,30] Vitamin E supplementation is used in many diseases in which ROS are thought to have a causative role.[31]

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MATERIALS AND METHODS

Subjects: The population studied consisted of 20 patients with active BD (aged 20 to 54 years, M/F ratio 9/11, mean age 37.9±10.3) diagnosed according to International Study Groups criteria, and 20 healthy volunteers (aged 28 to 53 years, M/F ratio 1, mean age 35.05±6.94) All the patients were considered to have the active form of the disease, on the basis of the presence of at least one of the major symptoms of BD. At the beginning of the study, all patients with BD were being treated with colchicine. Colchicine therapy was stopped for two weeks and basal blood samples were taken at the end of this treatment-free period. Patients with BD were divided into two groups. Group 1 comprised 10 patients with BD who were given colchicine 1.5 mg/day for three months. Group II comprised 10 patients with BD who were given the same dose of colchicine plus vitamin E 400 IU/day. Disease manifestations of the patients with BD are shown in Table 1 (only positive findings were considered). Group III comprised 20 volunteers who had no clinical or laboratory evidence of any disease and were included in the study as the control group.

In all subjects, the levels of plasma MDA and the activities of SOD and GSH-Px in erythrocytes were measured. Blood samples were taken from an antecubital vein from fasting subjects at the beginning of the study and after the treatments.

Before the study all subjects were checked hematologically and biochemically, and those with normal results were recruited.

All subjects gave informed, written consent. In all patients the treatments were well tolerated and no patients were withdrawn from the study because of adverse effects.

Blood parameters: Blood samples were obtained in the morning following an overnight fast. Ethylenediaminetetraacetate (EDTA) blood was centrifuged at 4000 rpm for 5 min at +4 °C, and plasma samples were transferred and stored at -80 °C until assay. The remaining erythrocytes were washed three times with 0.9% NaCl. Subsequently erythrocytes were lysed with cold distilled water. Erythrocyte lysates were stored at -25 °C until assay.

Plasma MDA Assay: The lipid peroxidation assay of blood samples was performed measuring Thiobarbituric Acid Reactive Substances (TBARS) formation as described by Kurtel et al.[32] The values of plasma MDA were expressed as μmol/L.

Erythrocytes SOD and GSH-Px activity: Erythrocyte SOD activity was assayed as described by Sun et al.,[33] and GSH-Px activity by Paglia et al.[34] as previously described by Köse et al.[12] The values of erythrocyte SOD and GSH-Px were expressed as U/g Hb.

Statistical analysis: Statistical Package for the Social Sciences 11.0 (SPSS 11.0) was used for statistical analysis. Three group comparisons were carried out using the Kruskal-Wallis U-test and two group comparisons were carried out using the Mann-Whitney U-test in the analysis of plasma MDA levels and erythrocyte SOD and GSH-Px levels. In pre-treatment and post-treatment comparisons, paired t tests were used. Values of $p<0.05$ were considered significant.

RESULTS

Sex and age: No statistically significant differences were observed between the three groups according to age or sex ($p>0.05$).

Plasma MDA levels: The initial plasma levels of MDA in Groups I, II, and III, and the levels after three months of colchicine and colchicine plus vitamin E are shown in Table 2. The mean initial levels of plasma MDA were 0.64±0.71, 0.75±0.75, and 0.30±0.17 in Groups I, II, and III, respectively. According to these values, plasma MDA levels showed no statistically significant difference between Groups I and II ($p>0.05$), but the levels were apparently higher than those in Group III ($p<0.05$).

The changes in MDA levels after three months of treatment are shown in Table 2. When compared to basal plasma levels, the post-treatment levels were not different in Groups I and II. Moreover, when compared to Group III, the statistical analysis of post-treatment plasma MDA levels in Groups I and II showed no statistically significant difference ($p>0.05$) (Table 2).

Antioxidant enzyme status: The initial erythrocyte levels of SOD and GSH-Px in Groups I, II, and III and the levels after treatment are shown in Table 2.

i. Erythrocyte SOD levels: The results in this table showed that initial erythrocyte SOD levels in Groups I and II were not statistically different ($p>0.05$) but both were significantly lower than those in Group III ($p<0.05$). The analysis of changes in erythrocyte SOD levels demonstrated that both therapies increased the SOD levels in BD patients ($p<0.05$). When compared to Group III, these post-treatment values of erythrocyte SOD did not show any difference and also they did not differ between Groups I and II ($p>0.05$) (Table 2).

ii. Erythrocyte GSH-Px levels: Table 2 shows that erythrocyte GSH-Px levels in Group I and Group II had similar values ($p>0.05$), which were lower than those in Group III ($p<0.05$). After therapy the erythrocyte GSH-Px levels showed no statistically significant differences between Groups I and II. Both treatments increased the erythrocyte GSH-Px levels ($p<0.05$ each). The post-treatment levels were also higher than those in Group III ($p<0.05$) (Table 2).

DISCUSSION

Our study has several implications: a) MDA levels in plasma were increased in active BD, b) erythrocyte antioxidant enzymes were reduced in active BD c) Colchicine and colchicine plus vitamin E therapy achieved a decrease in serum MDA and an increase in erythrocyte antioxidants, and d) the amount of changes in the oxidant/antioxidant system did not differ in the treatment groups.

In the current study, plasma MDA levels of patients with BD were higher than those of the healthy controls, in agreement with previous reports.[11-13,16,23] This is important as it confirms the presence of increased ROS in the disease. After three months of therapy, plasma MDA levels were decreased. We are not aware of any controlled study on the effects of colchicine on lipid peroxidation or MDA levels in patients with BD.

Colchicine was found to block the oxygen generation in leukocytes stimulated with opsonized zymosan and superoxide scavenging activity of leukocytes was significantly higher in BD patients receiving colchicine than in those not undergoing such treatment.35 By inhibiting phagocytosis of leukocytes, colchicine prevented the production of ROS.14 These effects may be involved in the beneficial effects of colchicine in the treatment of patients with BD.[3,35,14]

Antioxidant effects of vitamin E have already been reported in the elderly,[36] smokers,[37] and uremic patients.[20,30,38] Vitamin E supplementation achieved a decrease in MDA levels in the above mentioned population. In addition, the effects of vitamin E supplementation on blood antioxidant levels in patients with BD were studied by Kökçam et al.[16] Similar to the current study, they found that vitamin E decreased plasma MDA levels in patients with BD. When compared to colchicine, vitamin E supplementation did not achieve great changes in the oxidant/antioxidant system in our patients with BD. This may be the result of lower levels of serum selenium and vitamin E in BD.[16,39] Selenium is important in the absorption of vitamin E. Lower levels could destroy the function of vitamin E in BD. Secondly, colchicine and vitamin E have different functions and when used together their effects may not be synergistic or additive.

Erythrocyte SOD levels in the patients with active BD were lower than those in the control group, in agreement with the current literature.[13,14] As it is an antioxidant enzyme, this shows that the antioxidant capacity in BD is decreased.[14] Decreases in the SOD level could be involved in the increase in ROS production and in the etiopathogenesis of the disease.[1,26,27] SOD treatment induced remission in patients with active BD. This therapeutic effect was previously thought to be related to the decrement of ROS[14,14] or the reduced influx of neutrophils to inflammation sites.40 The reasons for the decrement of SOD levels may be as follows: a suppression in SOD synthesis due to a genetic effect, leakage of SOD out of the cell due to increased production of oxygen radicals causing membrane damage, or inactivation of SOD by the increased H2O2 production in the cell.[15] Decreases in the antioxidant levels in erythrocytes may be related to insufficient superoxide scavenging activity.[17,35]

In the literature, increased SOD levels were found in the serum[24.41] and erythrocytes[12,24] of patients with BD. Finding that the rate of erythrocyte turnover was higher in BD, Köse et al. postulated that increased SOD activity reflected the appearance of young erythrocytes having high levels of SOD in these patients.[12]

GSH-Px levels in patients with BD have been studied in erythrocytes, neutrophils, and plasma. Most of them have conflicting results.[12,14,16,26,27] Erythrocyte GSH-Px levels in patients with active BD were lower than those in the control group in the current study and this was in agreement with previous studies.[12.14.16.26] The possible factors that contribute to the decrement of GSH-Px activity in patients with BD may be i. Inhibition of GSH-Px activity in the presence of oxidative stress;[15] previously superoxide anion was shown to inhibit the function of GSH-Px[42]. ii. Patients with BD may have lower selenium and thiol levels or may have genetic polymorphism that may affect the activity of the enzyme.[15,21,39] iii. The negative correlation between MDA and GSH-Px activities; it is thought that toxic ligands like MDA could inhibit the activity of GSH-Px.[12]

An antioxidant defense system can be achieved by either increases in the activities of some enzymes such as GSH-Px and SOD or preventing the production of superoxide radicals. When oxidative stress occurs in the organism, the antioxidant enzyme activity begins to increase and prevents tissue injury.3,25 Increased oxidant stress and decreased antioxidant enzymes activities together may give rise to tissue inflammation or to endothelial damage observed in BD. The other mechanisms that contribute to the pathogenesis of BD are the suppression of antioxidant enzymes and the reduced production of components of the antioxidant system.[14]

Three months of therapy with colchicine and colchicine plus vitamin E resulted in a significant increase in erythrocyte SOD and GSH-Px activities. There is only one study in the literature that was comparable to our results. Kökçam et al.[16] investigated the effects of vitamin E supplementation on blood antioxidant parameters in patients with BD. Both studies found that vitamin E supplementation achieved an increase in GSH-Px activity in BD. However, they also found a dramatic increase in the enzyme activity, which we could not replicate. The reason for this difference may be the higher doses of vitamin E supplementation given in their study or the preference of giving vitamin E alone. The optimum dose or the appropriate duration of the vitamin E supplementation for BD has not been defined. According to the results, the increased production of free radicals in BD, as shown by higher MDA levels and lower SOD and GSH-Px activities, may provide some evidence for oxidant/antioxidant system impairments in BD, which favored the oxidation mechanisms seen in the disease.

The effects of colchicine on these systems may contribute to its beneficial effects in BD. In conclusion, our results suggest that 400 IU/day vitamin E did not potentiate the beneficial effect of colchicines on antioxidant defense systems in BD.

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