Superoxide Dismutase and Glutathione Peroxidase in Behçet's Disease

BEHÇET HASTALIĞINDA SUPEROKSİD DİSMUTAZ VE GLUTATYON PEROKSİDAZ AKTİVİTESİ

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SUMMARY

It has recently been suggested that excessively generated free oxygen radicals also play a role in the pathogenesis of various diseases. In this study, Superoxide Dismutase (SOD) and Glutathione Peroxidase (GSH-Px) activities were measured in erythrocytes in order to find out the role of oxidative stress in the etiopathogenesis of Behçet's Disease.

The study was performed on 33 patients with Behçet's Disease and age and sex-matched 37 healthy control subjects. It was found that the SOD activities in patients with Behçet's Disease were significantly lower than those of controls (178.65 ± 45.73 mg/ml vs. 200.92 ± 44.68 mg/ml, (p< 0.05). Although the GSH-Px activities in those patients were lower than those of the control subjects, this difference wasn't statistically significant (2.9427 ± 0.892 U/ml. vs. 3.1051 ± 0.713 U/ml., p> 0.05). When SOD and GSH-Px values were compared in active and inactive patients with Behçet's Disease, no significant difference was noted.

Consequently, excessive amount of free oxygen radicals may be responsible for the etiopathogenesis of Behçet's Disease or at least they may contribute to it. This is possibly due to diminished SOD activity but further studies are required to find out the exact role of SOD and GSH-Px activities in the etiopathogenesis of Behçet's Disease.

Key Words: Behçet's Disease, superoxide dismutase, glutathione peroxidase

ÖZET

Çeşitli hastalıkların patogenezinde aşırı miktarda oluşan serbest oksijen radikallerinin önemli rol oynadığı kabul edilmektedir.

Bu çalışmamızda Behçet Hastalığı etyopatogenezinde oksidatif stresin rolünü tayin etmek amacıyla eritrositlerde Süperoksid Dismutaz (SOD) ve Glutatyon Peroksidaz (GSH-Px) aktivitelerini ölçtük. Çalışma yaş ve cinsiyet bakımından uygun, 33 Behçet hastası ve 37 kontrol olgusu ile yapıldı.

Behçetli hastalarda SOD aktivitesinde (178.65 ± 45.73 mg/ml), kontrol grubuna göre (200.92 ± 44.68 mg/ml) anlamlı bir düşüklük tespit edildi (p< 0.05). Bu hastalarda GSH-Px aktivitesi (2.9427 ± 0.892 U/ml), kontrol grubuna (3.1052 ± 0.713 U/ml) göre düşük tespit edilmesine rağmen, bu düşüklük istatistiksel olarak anlamlı değildi (p> 0.05). Aktif ve inaktif Behçetli hastaların SOD ve GSH-Px değerleri karşılaştırıldığında istatistiksel olarak anlamlı bir fark yoktu.

Sonuç olarak; Aşırı miktarda oluşan serbest oksijen radikalleri Behçet Hastalığı etyopatogenezinden sorumlu olabilir veya en azından katkıda bulunabilir. Bu durum muhtemelen SOD aktivitesindeki azalmaya bağlıdır. Buna karşılık Behçet Hastalığı etyopatogenezinde SOD ve GSH-Px aktivitelerinin kesin rolünü tayin etmek için daha geniş çaplı araştırmalara gereksinim vardır.

Anahtar Kelimeler: Behçet Hastalığı, süperoksit dismutaz, glutatyon peroksidaz

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Reactive free oxygen radicals can damage DNA, lipids, proteins and carbonhydrates resulting in several diseases.

Oxydative stress at different degrees is present in many diseases. The important part how much the pathogenesis in affected. Pronai et al. (1) reported that oxydative products like superoxide radikals were possible mediators on tissue damage in Behçet's disease supports this theory (2,3).

In general the enzymes like SOD and GSH-Px prevents the organism from the damage of free radicals.

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These enzyme systems increase their activities and help keep their protective effect in case of high oxydative stress condition. It's reported that there are metabolic changes in some of the enzymes and element in those enzymes take part in oxydative stress (1,4,5) in Behçet's disease. Hence, the activities of erythrocyte enzymes which had antioxydative effect (SOD, GSH-Px) were investigated in this study to determine the role of oxydative stress on Behçet's disease.

MATERIAL AND METHOD

This study was performed on 33 patients with Behçet's disease diagnosed in Gülhane Military Medical Academy (GMMA) Internal Medicine Department between January 3, 1995 and September 20, 1995. 3 of the cases were females (9%) and 30 of them were males (91%). The mean age was 29.5 ± 4.65 years (range 21-64 years). The range of disease onset of cases was between 5 months and 24 years. The diagnoses were determined according to the criteria of "International Behçet's Working Group". 11 out of 33 patients were active and 22 out of 33 were inactive patients. The patients having at least two of the signs oral ulcer, genital ulcer, eye lesions or arthritis and those having high C-reactive protein and erythrocyte sedimentation levels are accepted as active patients. Of our cases 24 had oral ulcers, 27 had genital ulcers, 11 had eye lesions, 18 had skin findings, 6 had joint involvement, 7 had thrombophylebitis, 2 had neurologic findings, 1 had intestinal and 1 had pulmonary involvements. Pathergy reaction was positive in 6 out of 33 patients (17.4%).

HLA B5 was positive in 6 out of 24 patients (27%) whose HLA typings were determined. There were two patients with history of Behçet's Disease. The most common symtom was oral ulcer in families of 11 patients. The numbers of patients treated with colchicine, cortison, immunosuppressive drugs and combination of these drugs were 8, 3, 1 and 9 respectively. 12 patients weren't administered any medication. Healty 37 subjects among hospital staff were accepted as the control group. 3 of them were females (8%) and 34 were males (92%). The mean age was 29.6 ± 1.48 years (range 18-62).

Preparation of extract of erythrocytes from blood samples were performed in GMMA Pharmacy Department and GMMA Internal Medicine Laboratories. Blood samples were placed in to tubes with EDTA and centrifugated for 5 minutes by 6000 rpm. After separating the plasma, tree times saline of the rest volume was put over the erythrocyte part. Following the centrifugation for 5 minutes in 6000 rpm the upper part of the suspension was poured. After doing this washing procedure three times, 1 ml of the erythrocyte part was taken and 4 ml pure water was placed on it and the erythrocytes were haemolysed, the results material were put in polypropylen tubes and kept until analysis.

After completing the blood samples of patients and control subjects, the enzyme analyses were done in GMMA Pharmacy Department. Erythrocyte (GSH-Px and SOD activities were studied using the methods described by Plepan (5) and Sun (6), respectively.

Analysis of data was done using Student's-t, Mann Whitney-U and X^2 tests. Results were determined with mean ± standart error.

FINDINGS

The features due to patients with Behçet's disease and control subjects and the values of SOD GSH-Px activities were shown on Table 3 and Table 4. There were not significant differences between patients with Behçet's disease and control subjects in terms of their age and sex evaluation (Table 1 and Table 2).

SOD and GSH-Px activities in the patients group were detected as 178.65 ± 45.73 mg/ml and 2.9427 ± 0.892 U/ml, respectively. SOD and GSH-Px

Table 1. The correlation of ages in patients and control subjects.

Group	Number of cases	Range of age	Mean of age	t	P
Patients with Behçet's Disease	33	21-64	29.5 ± 1.65	0.027	>0.05
Control subjects	37	18-62	29.6 ± 1.48		

Table 2. The correlation of sex in patients and control subjects.

Group	Male	Famale	Total	X^2	P
Patient Group	30	3	33		
Control Group	34	3	37	0.327	>0.05
Total	64	6	70		

Table 3. The correlation of SOD activities in patients and control subjects.

	Number of Cases	SOD ACTIVITIES (Mg/ML)	t	P
Active Patients	11	173.85 ± 55.07		
Inactive Patient	22	208.32 ± 13.06	patient x control: -2.0558	<0.05
Patient Group	33	178.65 ± 45.73	Active x inactive: 0.2288	>0.05
Control Group	37	200.92 ± 44.68		

Table 4. The correlation of GSH-Px activities in patients and control subjects.

	Number of Cases	SOD ACTIVITIES (Mg/ML)	t	P
Active Patients	11	3.127 ± 0.982		
Inactive Patient	22	2.850 ± 0.853	Patient x control: -0.8355	>0.05
Patient Group	33	2.9427 ± 0.892	Active x inactive: 0.3687	>0.05
Control Group	37	3.1052 ± 0.713		

activities in the control group were detected as 200.92 ± 44.6 mg/ml and 3.1052 ± 0.7 U/ml, respectively.

SOD activity in the patient group was lower significantly that the activity in the control group ($p< 0.05$). Although GSH-Px activity in the patient group was lower that that of the control group, it was not significant statistically ($p> 0.05$) (Table 3 and 4).

SOD and GSH-Px activities in patients with active Behçet's disease and SOD and GSH-Px activities in those with inactive disease were found 173.85 ± 55.07 mg/ml, 2.127 ± 0.982 U/ml and 208.32 ± 13.06 mg/ml 2.850 ± 0.853 U/ml respectively. SOD activities in active group were lower than those in inactive group significantly ($p< 0.05$).

Although SOD activities in inactive patients were higher that the control subject, it was not significant statistically ($p> 0.05$). The correlation of GSH-Px activities between active or inactive patients with control subjects didn't show any statistically significant difference ($p> 0.05$). SOD and GSH-Px activities were not different significantly in active and inactive patients ($p> 0.05$) (Table 3 and Table 4).

DISCUSSION

Free radicals and the other reactive oxygen types are formed continously in human body. Most of them have useful physiologic functions. They can be toxic only when produced excessively. This toxicity increases particularly with the association of the metals like Fe^{++} and Cu^{++}. Reactive free oxygen radicals effect reversibly or irreversibly several biochemical reactions consisting of nucleic acids, proteins, aminoacids, lipids, lipoproteins, carbohydrates and some tissue macromolecules. Free radicals demolish cell function. due to the exposure time and quantity (7,8).

One or more of these biochemical changes may be responsible from several diseases. Oxydative stress is patients in most of diseases more or less. The important part is how much it effects the pathogenesis. Antioxydation defence system affect biologically by cleaning important reactive oxygen radicals, by preventing their forming or by reparing the damages they cause (9). In general the enzyme systems like SOD and GSH-Px protect the organism from damages which free radicals may causes. These enzyme systems increase their activities and try to keep their protective effect in case of high oxydative stress situation.

The inequilibrium between oxygen radicals and antioxydation defense system may cause frequently oxydative stress that results in metabolic damage and cell death. Oxydative stress may arouse because of the deficiency of glutathione, ascorbat, and αtocoferol or the diminished activity of SOD; GSH-Px and CAT or increased production of free oxygen radicals (10).

There has been several reports claiming the positivity of the pathergy test depends on both the sharpness of the needle used and the antiseptic material applied for dysinjection of the skin. 6 of 33 patient (17%) involved in our study had positive result for the pathergy test (11-14).

Niwa et al. (3,16) reported that the effect of oxygen radicals on tissue damage was more than that of lysosomal enzymes in Behçet's disease. Pronai et al (1) found that the activity of superoxyde cleaning of PMN leucocytes was lower significantly in patients with Behçet's disease than that in healthy subject and they

reported that the decrease in this activity may be the cause of increasing superoxyde production.

Jiang et al. (15) stated that the excessive production of free radicals in patients with systemic lupus erythematosis may play an important role in pathogenesis and this may be related to the decrease in the activity of SOD. Youssef et al. (17) found high SOD activities of YMN leucocytes in patients with rheumatoid arhritis. Tarp et al (18) found low erythrocyte GSH-Px activities in these patients. In our study, we haven't look for considered rheumatoid arthritis.

There haven't been so many reports on SOD and GSH-Px activities in patients with Behçet's disease in the literature yet. We investigated these activities which were indicators of oxydative stress in Behçet's disease. SOD activity in patients with Behçet's disease was found lower significantly than that in healthy subjects. Also GSH-Px activities weren't different when compared between patients with active and inactive Behçet's disease. It means that SOD and GSH-Px levels do not change related to diseases and their stages. So these levels are not useful to diagnose and to determine the activity.

As a results, although free oxygen redicals increase in Behçet's disease the relative insufficiency of SOD may be seen or whether free oxygen radical increase or not the production of SOD may be low again. Our study support the second theory. To determine exactly, it is necessary to investigate with more subjects and more parameters. The same study should be done also in plasma where these enzym activities are seen and investigated the effects of trace elements.

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