Superoxide Dismutase and Glutathione Peroxidase in Behçet’s Disease

BEHÇET HASTALIĞINDA SUPEROKSID DİSMUTAZ VE GLUTATYON PEROKSIDAZ 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 pathogenesis 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 miktarlı oksijen radikallerinin önemli rol olduğu kabul edilmektedir.

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

Behçetli hastalarında SOD aktivitesinde (178.65 ± 45.73 mg/ml), kontrol grubuna göre (200.92 ± 44.68 mg/ml) anlamlı bir düşüş 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 düşüş tespit edilmesine rağmen, bu düşüş istatistiksel olarak anlamlı değildir (p> 0.05). Aktif ve inaktif Behçetli hastaların SOD ve GSH-Px değerlerinin karşılaştırıldığında istatistiksel olarak anlamlı bir fark yoktu.

Sonuç olarak; Aşırı miktarlı oksijen serbest oksijen radikalleri Behçet Hastalığı etiopatogenezinde sorumlu olabilir ve en azından katkıda bulunabilir. Bu durum unutmamalı ve bu durum tedavi edilmesi gerektir. Buna karşılık Behçet Hastalığı etiopatogenezinde SOD ve GSH-Px aktivitelerinin kesin rolünü tayin etmek için daha geniş çaplı araştırmalar gereklidir.

Anahtar Kelimeler: Behçet Hastalığı, süperoksit dismutaz, glutatyon peroksidaz


Oxidative stress at different degrees is present in many diseases. The important part how much the pathogenesis in affected. Pronai et al. (1) reported that oxidative products like superoxide radicals 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 oxidative stress condition. It’s reported that there are metabolic changes in some of the enzymes and element in those enzymes take part in oxidative stress (1,4,5) in Behçet’s disease. Hence, the activities of erythrocyte enzymes which had antioxidative effect (SOD, GSH-Px) were investigated in this study to determine the role of oxidative 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 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 thrombophlebitis, 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 symptom 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. Healthy 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 polypropylene 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 X² 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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases</th>
<th>Range of age</th>
<th>Mean of age</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with Behçet’s Disease</td>
<td>33</td>
<td>21-64</td>
<td>29.5 ± 1.65</td>
<td>0.027</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Control subjects</td>
<td>37</td>
<td>18-62</td>
<td>29.6 ± 1.48</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Group</td>
<td>30</td>
<td>3</td>
<td>33</td>
<td>0.327</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Control Group</td>
<td>34</td>
<td>3</td>
<td>37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>6</td>
<td>70</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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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 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 173.65 ± 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 continuously 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 Fe²⁺ and Cu²⁺. Reactive free oxygen radicals effect reversibly or irreversibly several biochemical reactions consisting of nucleic acids, proteins, aminoacids, lipids, lipoproteins, carbohydrates and some tissue macro-molecules. 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. Oxidative stress is patients in most of diseases more or less. The important part is how much it effects the pathogenesis. Antioxidation defense system affect biologically by cleaning important reactive oxygen radicals, by preventing their forming or by repairing 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 oxidative stress situation.

The inequilibrium between oxygen radicals and antioxidation defense system may cause frequently oxidative stress that results in metabolic damage and cell death. Oxidative stress may arouse because of the deficiency of glutathione, ascobrat, 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 superoxide cleaning of PMN leukocytes was lower significantly in patients with Behçet’s disease than that in healthy subject and they

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

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

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

<table>
<thead>
<tr>
<th></th>
<th>Number of Cases</th>
<th>SOD ACTIVITIES (Mg/ML)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active Patients</td>
<td>11</td>
<td>3.127 ± 0.982</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactive Patient</td>
<td>22</td>
<td>2.850 ± 0.853</td>
<td>Patient x control: -0.8355</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Patient Group</td>
<td>33</td>
<td>2.9427 ± 0.892</td>
<td>Active x inactive: 0.3687</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Control Group</td>
<td>37</td>
<td>3.1052 ± 0.713</td>
<td></td>
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reported that the decrease in this activity may be the cause of increasing superoxide production.

Jiang et al. (15) stated that the excessive production of free radicals in patients with systemic lupus erythematosus 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 leukocytes in patients with rheumatoid arthritis. 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 oxidative 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 radicals 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 enzyme activities are seen and investigated the effects of trace elements.

REFERENCES