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Paraoxonase activities and erythrocyte TBARS levels in patients with Behçet's disease and recurrent aphthous stomatitis

Behçet hastalığı ve rekkürren aftöz stomatitli hastalarda paraoksonaz aktivitesi ve eritrosit TBARS düzeyleri

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Summary

Aim: The aim of our study is to evaluate paraoxonase (PON1), superoxide dismutase (SOD) and catalase (CAT) activities which is antioxidant enzymes, and tiobarbituric acid substance (TBARS) levels, one of the end products of lipid peroxidation induced by reactive oxygen species, in patients with Behçet's disease (BD) and Recurrent Aphthous Stomatitis. We also aimed to investigate if paraoxonase phenotyping at position 192., may prone to affect to prone to Behcet's disease and Recurrent Aphthous Stomatitis or not.

Materials and Methods: Eryrocyte SOD, CAT activities and TBARS levels, serum PON1 activities were measured by using spectrophotometer. Individuals were classified for PON1 phenotype using the antimode of histogram of PON1 as proposed by Eckerson.

Results: Erytrocyte TBARS levels and SOD activities of Behcet's disease group higher than controls (p=0.05). While TBARS levels depleted in patients under treatment with colchicines, no effect was observed on the antioxidant enzyme activities by colchicine treatment in Behcet's disease patients (n=10). We also investigated if paraoxonase phenotyping affected to prone to Behcet's disease. While 18% of patients with Behcet's disease had BB phenotype, 13,6% of controls.

Conclusion: Although our data can not exactly indicate that B allele carriers are more prone to BD and/or any chronic inflammatory disease, taking into account this preliminary study with limited number of cases, we can propose that B allele carriers may have a tendency for these diseases, and further population studies on this allele frequency related with these diseases are needed.

Key Words: Paraoxonase, Behçet's Disease, Recurrent Aphthous Stomatitis, superoxide dismutase, catalase.

Özet

Amaç: *Bu çalışmanın amacı Behçet hastaları ve rekküren aftöz stomatitli (RAS) hastalarda antioksidan enzimlerden süperoksid dismutaz (SOD), katalaz (CAT) ve paraoksonaz (PON1) ile lipid peroksidasyonunu gösteren tiyobarbitürik asidle ilişkili substrat (TBARS) düzeyleri yanı sıra 192. pozisyonda paraoksonaz fenotipini belirleyerek, hem Behçet ile rekküren aftöz stomatitli hastalarda antioksidan enzimlerin durumunu tespit etmek hem de 192. pozisyonda paraoksonaz fenotipinin Behçet Hastalığına bir yatkınlık yaratıp yaratmadığını belirlemektir.*

Gereç ve Yöntem: *Bu amaçla 25 Behçet hastası, 27 RAS'li hasta ile 45 sağlıklı gönüllü çalışmaya dahil edilmiştir. Eritrosit SOD, CAT, PON1 enzim aktiviteleri ve TBARS düzeyi spektrofotometrik olarak, 192. pozisyonda PON1 fenotipi Eckersonun önerdiği PON1 histogram antimode kullanılarak belirlenmiştir.*

Bulgular: *Eritrosit TBARS ve SOD enzim aktiviteleri Behçet hastalarında kontrole göre anlamlı yüksek bulunmuştur (p=0.05). Kolşisin tedavisi alan Behçet hastalarında (n=10) TBARS düzeyleri düşerken, antioksidan enzimlerde bir değişiklik gözlenmedi. Behçet hastalarının %18'i, kontrollerin %13,6'sı BB fenotipi taşıyordu.*

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Sonuç: *Kolşisin tedavisinin eritrosit TBARS düzeylerini düşürmesi nedeniyle, Behçet hastalığının patofizyolojisinde polimorfnükleer lökositlerin serbest radikallerin temel kaynağını oluşturmaktadır. Ayrıca çalışmamız BB alleli taşıyan olguların Behçet Hastalığına yatkınlığının daha fazla olabileceğini gösteren bir ön çalışma niteliğindedir. Ancak olgu sayısının arttırılarak bu konuda daha ileri çalışmalara gereksinim bulunmaktadır.*

Anahtar Kelimeler: *Paraoksonaz, rekkürren aftöz stomatit, Behçet Hastalığı, süperoksid dismutaz, katalaz.*

Introduction

Behçet's Disease (BD) is a chronic inflammatory, multisystemic disease characterized by oral, genital ulcerations and uveitis. Since its first description in 1937 by Hulusi Behçet, additional features and complications of the syndrome have been reported, however oral ulcerations have been the mandatory manifestation in all these reports (1,2). A lot of research also investigated the various immunologic, viral, toxic and genetic abnormalities underlying the pathophysiology of this chronic, inflammatory disease. Although the exact underlying mechanism is still unknown, recent findings on the deficient antioxidant system in the active stage of these patients have attracted attention (3-6). Recurrent aphthous stomatitis (RAS) is a frequent disease characterized by recurrent oral aphthae without systemic involvement. Local trauma, certain foods, emotional stress, hormonal changes, microbial agents and immunogenetic predisposition have also been implicated in this disease but the exact pathogenesis is still not known (7).

Superoxide dismutase (SOD) is a family of enzymes, comprising CuSOD, ZnSOD, MnSOD and extracellular SOD, whose function is protection against reactive oxygen species (ROS), particularly the superoxide anion. Catalase (CAT) is also an important factor in oxidative defence and plays a major role in the detoxification of hydrogen peroxide (8).

ROS can attack double bonds in polyunsaturated fatty acids, and thus induce lipid peroxidation, which in turn results in further oxidative damage. ROS mediated oxidation of cell membrane lipids leads to the formation of lipid peroxidation products, such as MDA (9).

Serum paraoxonase (PON1) is a 45-kDa glycoprotein and prevents LDL oxidation by removing oxidised phosholipids from LDL. The genetic basis of the inter-individual variability of PON1 activity has been attributed to the presence of an A-G polymorphism in the coding region of the gene coding for this enzyme (10, 11). The A/G polymorphism corresponds to glutamine/arginine polymorphism at amino acid position 192. Individuals homozygous for arginine at position 192 (B genotype) show a significantly higher serum PON1 activity than those homozygous for Gln (A genotype) (10, 11, 12, 13). There are some studies showing that PON1 activity is exhausted during the BD active stage while fighting increased free radicals (14). Studies related with PON1enzyme activity had been performed in Behçet patients but there no PON1 phenotype investigation in patients with recurrent aphthous stomatitis as of yet.

The aim of our study is to evaluate PON1, SOD and CAT activities which are antioxidant enzymes, and tiobarbituric acid substance (TBARS) levels, one of the end products of lipid peroxidation induced by reactive oxygen species, in patients with BD and RAS. We also aimed to investigate whether or not PON1 phenotyping at position 192, is prone to BD and RAS.

Materials and Methods

The study was conducted on patients who were admitted and/or were being followed up (under the diagnosis of BD) by the Dermatology Outpatient Clinic. Behçet patients (n=25) were diagnosed by the criteria of the International Study Group for Behçet's Disease (1). Briefly, recurrent oral ulceration plus two of recurrent genital ulceration, eye lesions, skin lesions, or positive pathergy test were needed for the diagnosis. The duration of disease ranged 1-20 years (mean: 5.94 years). Ten patients were receiving colchicine therapy (in a dose of 1.5 mg daily) at the time of the study.

RAS cases (n=27) were chosen among patients who were admitted to the Dermatology Outpatient Clinic. They were diagnosed as having RAS according to several criteria; such as minor aphthous, major aphthous or herpetiform ulceration observed by dermatologist at least three times in 12-month period. These cases were not receiving any treatment nor did they have any other symptoms of BD. No patients, neither those with BD nor those with RAS had any diseases such as diabetes mellitus, coronary heart disease, hypertension, cancer or any other disease affecting antioxidant enzyme activities.

The control group (n=45) comprised age and sex matched healthy voluntary health staff.

All reagents were purchased from Sigma Chemical Co (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

After overnight fasting, blood samples were drawn from the subjects. They were centrifuged immediately and the serum and plasma samples were kept at $-80^{o}C$ until assaying.

Preparation of haemolyzates: After centrifugation heparinised blood samples, haemolysates which were prepared as previously described by Sözmen et al. (7),

were used immediately for determination of SOD CAT activities and TBARS levels. *The haemoglobin values* were measured by Drabkin's method.

SOD activities were measured according to Sözmen et al. (15) based on the inhibition of autoxidation epinephrine by SOD at 480 nm, with a LKB Ultraspec 2 spectrophotometer (LKB Biochrom Ltd, Cambridge, England).

CAT activities were also determined as described by Sozmen et al.(15) in which the degradation of peroxide is recorded spectrophotometrically at 240 nm.

TBARS levels were performed by incubation with TBA solution for 30 min at 95° C 30 min (12).

Serum PON1 activities were assayed with or without addition of NaCl (1M) as basal and salt-stimulated activity (16). Individuals were classified for PON1 phenotype using the antimode of a histogram of PON1 as proposed by Eckerson (17).

Other serum parameters (total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol) were determined by routine laboratory methods using a Hitachi 705 autoanalyser.

Statistical Analysis

All statistical analysis were performed by the statistical package SPSS for Windows, version 10.0 (SPSS, Chicago, IL). Correlation was calculated as Spearman correlation coefficients. For comparison of means, the Newman-Keulls multiple range test and Mann Whitney U test were used to analyse the results. For distribution of allele frequencies between the groups, the chi-square test was used. In order to determine the risk ratio, a likelihood test was performed.

Results

Table-1 shows the baseline data obtained from all groups. There was no significant difference between the groups for all these parameters.

Table-1. The demographically characteristic of subjects. The data were presented as mean±SD. RAS: Recurrent Aphthous Stomatitis, BD: Behcet's Disease.

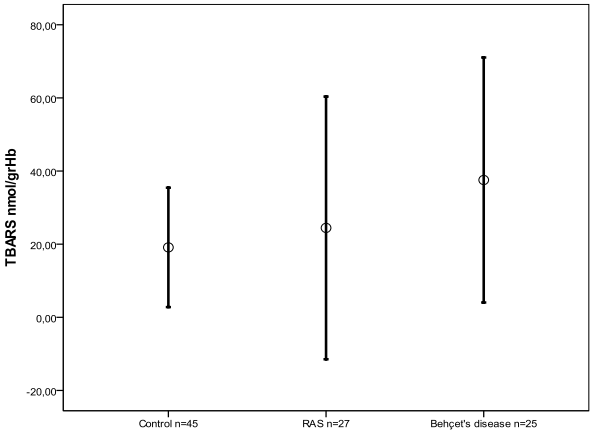
	Control group n=45	**RAS group n=27**	**BD group without treatment n=15**	**BD group Under treatment n=10**
Age	38.0 ± 9.2	34.5 ± 13.6	32.5 ± 12.0	38.2 ± 8.7
Gender (male/female)	16/29	10/17	8/7	6/10
Trygliceride (mg/dl)	111 ± 41	132 ± 22	140 ± 21	131 ± 24
T.cholesterol (mg/dl)	186 ± 39	176 ± 21	192 ± 18	194 ± 41
HDL-cholesterol (mg/dl)	54.7 ± 7.3	56.8 ± 4.9	55.4 ± 5.5	54.6 ± 6.1
LDL-cholesterol (mg/dl)	110 ± 35	92 ± 19	108 ± 19	100 ± 29

As seen in Figure-1, TBARS levels of the BD group were higher than the controls (p=0.05). Although PON1 activities of the BD group seem lower than the controls, this depletion was not found to be statistically significant.

SOD and CAT activities of all groups are presented in (Table-2). While CAT activities did not show any significant change between the groups, SOD activities of patients with BD were higher than controls (p=0.05).

When we evaluated the data based on whether or not they received the medications (mainly colchicine), we observed an increase in PON1 activities (25.5±30.2 vs 48.0±16.8, p=0.038) and a depletion in MDA levels (46.1±15.4 vs 20.5±8.9, p=0.028) of treated patients versus untreated patients (Figure-1 and Table-2).

Figure-1. PON1 activities and TBARS levels for all groups. MDA levels of BD were significantly different from controls (p=0.05) by Mann-Whitney U non parametric test.



RAS; Recurrent aphtous Stomatitis, BD; Behcet's disease without treatment

Table-2. Catalase (CAT) and superoxide dismutase (SOD) activities for all groups.

	Control group n=45	RAS group n=27	BD group n=25
SOD (U/gHb)	1234 ±1165	1373±771	2275±1724*
CAT (U/gHb)	6418±2218	7466±2351	5818±2004

Of the total, 18% of patients with BD and 13,6% of controls had BB phenotype. B allele frequencies were 0.25, 0.17 and 0.40 of controls, RAS and Behçet's patients, respectively. There was no significant difference in the distribution of allele frequencies between the groups. The effect of PON phenotype was not found to be significant in development of BD (p=0,676, likelihood ratio=2,326).

Discussion

We determined the TBARS levels and the antioxidant enzyme activities namely SOD, CAT and PON1 in patients with RAS and BD comparing to controls to evaluate if these enzyme activities might be used as a predictor for further BD. While the TBARS levels of the BD group were higher than the control and RAS groups (Figure-1), PON1 activities showed an insignificant depletion. In accordance with our findings, some authors (4,6,18) showed an increase in TBARS levels and a decrease in GSH-Px activity in BD. Our data support the hypothesis on the enhancement of oxygen radical formation in pathophysiology of BD. A depletion in PON1 activity in BD patients which was shown in this study, reflects new data on this subject. However, while SOD activity was higher in BD patients than in the controls, antioxidant enzyme activities in patients with RAS were similar to those of controls, contrary to our expectations. Since Whatelet et al. (2) determined that a simple aphthous ulceration which starts in a different period of life is the first clinical finding in BD, we had concluded that patients with RAS are more prone to BD in their future life and they might show similar changes in antioxidant enzyme activities and TBARS levels with BD.

An interesting finding of this study was depletion in TBARS levels through treatment with colchicine, a potent inhibitor of phagocytosis. It has been known that polymorphonuclear leukocytes (PMN) were the main source of free radicals in plasma and this data supposed that this source of free radical formation has a significant role in pathophysiology of BD. The data from some authors (5,14) are also in agreement with this result, as they also showed an increase in oxygen free radicals in PMN in the active stage of BD.

Interestingly, we also assessed a significant increase in PON1 activity in patients under treatment. It is known that PON1 protects LDL particles from oxidative damage and is proposed as an antioxidant enzyme against radical damage of endothelial cells (19). Some recent studies suggested that PON1 activity was low in patients with NIDDM, after myocardial infarction and active stage of BD (14,16,21). Tissue damage in BD is caused by the associated vasculitis and immune complex deposition within the blood vessel wall, together with an increase in CRP and leukocytes (22). Atherosclerosis is also related to an increase in inflammatory processes determined by an increase in CRP and other inflammatory substances (23). In light of this and our data, we can say that the underlying mechanism of clinical findings in BD were similar to atherosclerosis, therefore PON1 might have a main role during this mechanism. To clarify the effect of PON1 activity in the pathophysiologic mechanism of BD, evaluation of serum PON1 concentration and prospective studies for RAS patients are needed, which may be a subject for further studies.

It is necessary to take into account the phenotype in addition to the activity of PON1 while evaluating the protective role of PON1. Because AA phenotype, homozygous for PON1, is more effective for protection of LDL from oxidation and its activity of metabolizing the lipid peroxides is high (11-13). There are studies reporting that B allele a carries risk for coronary artery disease (CAD) in the Caucasian population (12). In our study, we aimed to investigate the PON1 activity and gene polymorphism at position 192. (Q-R) for the probable effects on BD and RAS. The results of this study showed that, of the total, 18% of patients with BD and 13.6% of controls had BB phenotype. Since there was no statistically significant correlation between the PON1 phenotype and BD by the chi-square test, the role of PON1 phenotype in the susceptibility to BD remains unclear. B allele frequency according to phenotype was 0.40 in BD and 0.25 in controls. This data revealed that B allele carriers are more prone to BD as well as a chronic inflammatory disease. Although there are a lot of conflicting results (24–27) related to PON1 phenotypes in a chronic inflammatory disease such as atherosclerosis, it is accepted that there is a relation between the B allele and CAD by most authors.

In conclusion, our data indicated that enhanced oxidative stress in BD patients causes an inhibition in PON1 activity and an increase in TBARS levels. Since PON1 activities increase, and TBARS levels decrease through inhibition of phagocytosis, we can say that inflammatory processes have a main role on the mechanism underlying of BD. As the data obtained from RAS group suggests, neither antioxidant enzymes nor TBARS levels

seem to be a predictive marker for BD. Although our data can not exactly indicate that B allele carriers are more prone to BD and/or any chronic inflammatory disease, taking into account this preliminary study with limited number of cases, we can propose that B allele carriers may have a tendency for these diseases, and further population studies on allele frequency related with these diseases are needed.

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