Asian Journal of Research in Biochemistry

Volume 14, Issue 5, Page 134-142, 2024; Article no.AJRB.124092
ISSN: 2582-0516

The Effect of Antioxidant Biomolecules in Ankylosing Spondylitis and Behçet's Diseases

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Authors' contributions

This work was carried out in collaboration among all authors. Author RB and RWD designed the study, authors EA and BD performed the statistical analysis, author ÇÇ wrote the protocol and performed assay, authors EA and EK wrote the first draft of the manuscript. Authors MAA and DA managed the analyses of the study. Author ÇÇ managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajrb/2024/v14i5319

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/124092

Original Research Article

Received: 16/07/2024
Accepted: 18/09/2024
Published: 23/09/2024

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Cite as: *Bilgin, Ramazan, Rana Waheed Dahham, Erkan Kozanoğlu, Burak Demir, Didem Arslan, Mehmet Ali Aşık, Çiğdem Çetin, and Ersin Akgöllü. 2024. "The Effect of Antioxidant Biomolecules in Ankylosing Spondylitis and Behçet's Diseases". Asian Journal of Research in Biochemistry 14 (5):134-42. https://doi.org/10.9734/ajrb/2024/v14i5319.*

ABSTRACT

Aims: Ankylosing spondylitis (AS) and Behçet's disease (BD) are both chronic inflammatory diseases of unknown etiology. It is thought that the development of both diseases is related to several factors such as environmental, genetic predisposition and oxidative stress. To control the flow of reactive oxygen species, aerobic cells have developed their own enzymatic and non-enzymatic defence system. The enzymatic ones are superoxide dismutase (SOD), catalase, glutathione peroxidase, non-enzymatic reduced glutathione (GSH). It is aimed to investigate the superoxide dismutase, malondialdehyde, catalase and reduced glutathione levels in patients with AS and BD in this study.

Study Design: This study consisted of the 34 patients with AS and 24 patients with BD and 20 healthy control subjects who applied to Çukurova University Faculty of Medicine Departments of Rheumatology and Department of Physical Medicine and Rehabilitation. Patients with haematological and other autoimmune diseases were excluded from the study.

Place and Duration of Study: Çukurova University Faculty of Medicine Departments of Rheumatology and Department of Physical Medicine and Rehabilitation and Arts & Science Faculty, Department of Chemistry (Biochemistry Division), Adana, Turkey between September 2022 and August 2024.

Methodology: In this study, superoxide dismutase (SOD), catalase, malondialdehyde (MDA), reduced glutathione (GSH) levels were determined by spectrometric methods for both groups and their values were compared with IBM SPSS 20 software.

Results: The median age was 46.34 in the Ankylosing spondylitis group and 38.50 in the Behçet's disease group, and 42.55 in healthy control group. 64.7% of the Ankylosing Spondylitis group, 62.5% of the Behçet's disease group and healthy control group 50% were male. While the SOD and MDA levels were found significantly higher in both groups according to control group, GSH and catalase levels were found to be lower and statistically significant in both groups compared to the control group.

Conclusion: We strongly recommend the inclusion of high-potential antioxidants that will strengthen the antioxidant defense mechanism and reduce peroxidation as a supportive medical therapy to patients with both diseases, especially for the patients with AS.

Keywords: Ankylosing spondylitis; Behçet's disease; superoxide dismutase; catalase; malondialdehyde; glutathione.

1. INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory disease of unknown etiology caused by inflammation of the spine and sacroiliac joints [1]. The development of AS is linked to a few factors such as environmental, sex, genetic predisposition, and oxidative stress. The exact factors that play a role in the pathogenesis of AS are not yet known. Most investigators reported that cytokines, chemokines (IL-27, IL-17) involved in autoimmunity, as well as reactive oxygen species (ROS) influence the pathogenesis of AS [2]. Increased oxidative stress plays a role in the rheumatoid arthritis, inflammatory bowel disease and ankylosing spondylitis [3]. Normally, cells have an oxidant/antioxidant balance in the physiological process. Insufficient antioxidant defence or increased production of ROS creates a condition known as oxidative stress as a result it may contribute to the development of AS [4].

Behçet's disease (BD) is a chronic disease that affects many systems of the body. The origin of the disease is unclear, though there are immune regulatory abnormalities in its pathogenesis [5]. Common symptoms of BD include oral aphthous and genital ulcers, skin lesions and uveitis. It is also rarely fatal, although symptoms related to the gastrointestinal tract, central nervous system and vascular system may occur. BD is seen worldwide, but there are geographical variations [6,7]. The BD is common in Silk Road countries from East Asia to the Mediterranean region. The incidence of the disease is 80-370 per 100,000 people in Turkey, while in Iran, Korea, China, and Oman are 13.5-20 [6,7].

To control the flow of ROS, aerobic cells have developed their own enzymatic and non-enzymatic defence system. While the enzymatic ones are superoxide dismutase (SOD), catalase, glutathione peroxidase, non-enzymatic one is reduced glutathione (GSH) [8]. SOD existing in

all cells that catalyses the reduction of superoxide free radicals to oxygen and hydrogen peroxide [8]. Malondialdehyde, which shows the oxidative transformation of polyunsaturated fatty acids, is an important indicator used in the determination of oxidative damage [9]. Free oxygen radicals and lipid peroxides play a role in the pathogenesis of many diseases including rheumatoid arthritis, systemic lupus erythematosus, infectious diseases, and atherosclerosis [9,10].

Oxidative stress occurs because of either an increase in the rate of formation or a decrease in the rate of elimination of free radicals. This situation shows a serious imbalance between free radical formation and antioxidant defense mechanisms and ultimately leads to tissue damage. In BD and AS, oxidative stress increases reactive oxygen species, and it slows down antioxidant defense mechanisms [11-13]. Therefore, the investigation of antioxidant enzymes in this kind of diseases is a vital issue.

In this study, the activities of antioxidant enzymes and the levels of related biomolecules in AS and BD patients were investigated using a control group.

2. MATERIALS AND METHODS

This study consisted of the patients with ankylosing spondylitis and Behçet diseases who applied to Çukurova University Faculty of Medicine Departments of Rheumatology and Department of Physical Medicine and Rehabilitation between March 2020 and June 2021. A total of 58 patients, including 34 patients with AS and 24 patients with BD and 20 healthy control subjects were enrolled in the study. The Ethics Committee of the Çukurova University has approved the study protocol. The participants in this study signed an informed consent form respecting the usage of their blood samples. This study was realized according to the Helsinki declaration declared in Edinburgh. Patients with haematological and other autoimmune diseases were excluded from the study. All subjects fasted after midnight before blood collection in the next morning. Blood samples of the patients were taken at the time of diagnosis, before starting the treatment. The samples were kept at minus 80 degrees until they were studied. In the next step, the levels of antioxidant molecules were determined from the plasma of the patients.

2.1 Determination of Superoxide Dismutase (SOD)

SOD catalyzes the dismutation of toxic superoxide radicals ($O_2^{\bullet -}$) into hydrogen peroxide and molecular oxygen. This process performs by reading the optical density (OD) given by the blue-coloured formazan dye formed using xanthine and superoxide radicals formed by using xanthine oxidase (XOD) with nitro blue tetrazolium (N.B.T) at a wavelength of 560 nm [14]. Briefly revised method, whole blood was centrifuged by 5000 g for 60 min, and 0.5 ml of obtained the supernatant was mixed with 2.45 ml buffer including 150 µmol/L NBT, 0.3 mmol/L xanthine oxidase, 0.6 mmol/L EDTA, 400 mmol/L Na_2CO_3 and 1 g/L bovine serum albumin. After incubation at 25 °C for 20 min, 0.5 ml of 0.8 mM $CuCl_2$ was added, and the measurement was performed at 560 nm.

2.2 Determination of Catalase Activity

CAT activities were measured by the procedure of Hadwan [15]. Briefly, reaction mixture was contained 2.5 mL of substrate made up of 10 mM hydrogen peroxide in a 50 mM phosphate buffer pH 7.0. Reaction was performed at 25 °C for 2 min and stopped by using 0.5 ml of 1 M HCl. The amount of H_2O_2 was defined by measuring its absorbance at 240 nm.

2.3 Determination of Reduced Glutathione (GSH)

Level of reduced glutathione (GSH) was measured according to the method proposed by Alisik [16]. Briefly, GSH was measured by determining the yellow-coloured complex formed by the conversion of 5,5′-dithio-bis 2-nitrobenzoic acid (DTNB) to 2-nitro-5-mercaptobenzoic acid in the plasma, which was measured by the spectrophotometer at 405 nm.

2.4 Determination of Malondialdehyde (MDA) Level

Malondialdehyde levels were determined according to the process proposed by Fauziah [17]. Briefly, MDA levels in plasma were measured with the Thiobarbituric–acid reaction. This reagent reacts with MDA and form a pink colour compound measured by fluorometry at 532 nm.

2.5 Statistical Analysis

Analyses was carried out using the IBM SPSS 20 software (USA). Statistics of data between both

groups were done the Mann-Whitney U test for continuous data, and chi-square test for categorical data. The statistical analyses of all enzyme's levels were performed with the Mann-Whitney U test because of they haven't got normal distribution. Since the erythrocyte, haemoglobin and haematocrit values of the patients had normal distribution, they were analysed with the student-t test. All enzymes' results were presented, and the median values were calculated because of 3 repetitions for each parameter.

3. RESULTS AND DISCUSSION

This study consisted of the patients with Ankylosing spondylitis and Behçet's diseases who applied to Çukurova University Faculty of Medicine Departments of Rheumatology and Department of Physical Medicine and Rehabilitation. A total of 78 participants, including 34 ankylosing spondylitis patients, 24 Behçet's patients and 20 healthy control persons were enrolled in the study. The median age was approximately 46 in the Ankylosing spondylitis group, 38 in the Behçet's disease group and 42 in control group. 64.7% of the Ankylosing Spondylitis group, 62.5% of the Behçet's disease group and 50% of control group were male. There were no differences between the groups in terms of age, gender, and complete blood cells (Table 1).

The mean follow-up period of patients with ankylosing spondylitis was 10.6 years. The mean of Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and The Ankylosing Spondylitis Disease Activity Score (ASDAS) scores were 5.1 and 3, respectively. HLA B27 was positive in 18 (52%) patients. Ankylosing spondylitis patients had arthritis in 10 (29%) patients, enthesitis in 16 (47%), uveitis in 3 (8%) patients, and inflammatory bowel disease in 1 (3%) patient. The mean follow-up period of the patients with Behçet's disease was 4.6 years. Behçet's patients had mucocutaneous involvement in 24 (100%) patients, uveitis in 6 (25%) patients, and vascular involvement in 3 (12.5%) patients. All patients were included in the study regardless of disease activity and organ involvement.

In this study, antioxidant enzymes and related-biomolecules superoxide dismutase, reduced glutathione, malondialdehyde and catalase levels were compared among Ankylosing spondylitis, Behçet's and control groups. Except for the GSH molecule, enzymes and MDA levels were different and statistically significant in both groups. Especially, SOD activity and MDA levels were found to be approximately 3-fold higher in patients with ankylosing spondylitis compared to patients with Behçet's disease (5.81 vs. 1.93 for SOD and 53.38 vs. 15.61 for MDA as mean values) (Table 1). In contrast, catalase enzyme activities were about 2-fold higher in cases with Behcet's disease compared to patients with ankylosing spondylitis (10.64 vs. 5.36 as mean value). When blood values were compared in both patient groups, no difference was detected (Table 1). Box plots of SOD, GSH, Catalase and MDA parameters in each group are given in Fig. 1.

In this study, for the first time, patients with AS and BD, and control group were compared in terms of the levels of antioxidant enzymes and antioxidant-related biomolecules. While Behçet's disease is mostly associated with vasculitis, ankylosing spondylitis is characterized by an involvement associated with inflammation of the axial skeleton and sacroiliac joints caused by autoinflammation [18,19]. The purpose of this comparison is to determine whether there is a significant difference between the levels of antioxidant enzymes and related biomolecules in both patient groups compared to control group, and thus to have an idea about the oxidant systems related to the etiopathogenesis of both diseases. A few researchers explored the levels of antioxidant enzyme and oxidation-related biomolecules on these two diseases [5,13, 19-23].

In the current study, SOD enzyme activity and MDA level were found to be much higher in patients with ankylosing spondylitis and Behçet's disease compared to control group. In contrast, catalase enzyme activities and GSH levels were lower in both patient groups compared to healthy control group. According to the results of the present study and literature data, it is seen that the oxidant/antioxidant mechanisms of people in these two disease groups are impaired. High levels of SOD and MDA, especially in patients with ankylosing spondylitis, indicate that superoxide radicals are more common. SOD catalyzes superoxide radicals to oxygen and hydrogen peroxide (H2O2) by dismutation reaction [8]. Studies have shown that the activity of this enzyme decreases, increases or remains at the same level comparison to controls in many inflammatory diseases such as Behçet's disease and AS [5,13,19-23]. High levels of SOD activity suggest that SOD is effective in the elimination of free oxygen radicals in inflammation [5,13]. In the present study, SOD activity was found to be very high compared to the control group, while

catalase activity, which will neutralise the hydrogen peroxide released after SOD catalysis, was found to be low. Under normal physiological conditions, catalase enzyme activity was expected to be high. Our present results show that the antioxidant/oxidant system is impaired in both ankylosing spondylitis and Behçet's disease.

In studies with Behçet's disease comparing a healthy control group, SOD levels were reported higher [5,13], lower [20,21] or insignificant [22,23]. In addition, there are studies reporting that MDA increased in the same patients compared to the controls [22,23]. Taysi et al. [5] reported that GSH and catalase levels were lower in individuals with BD than in the control group.

There are studies reporting that while SOD enzyme levels decrease in patients with ankylosing spondylitis, MDA biomolecule levels increase according to healthy people [2,24]. In another study, a decrease in catalase enzyme level was reported [19]. Ozgoçmen et al. [3] found that the level of catalase increased in AS disease. Additionally, Ozgoçmen et al. [3] reported that SOD enzyme levels did not differ between AS patients and healthy people.

The levels of antioxidants in tissues and organs are also different because of metabolic activity. However, it is certain that in both diseases, this oxidant/antioxidant mechanism is impaired compared to healthy individuals [8,18,19]. This deterioration varies according to the severity of the disease, the type of disease, and the tissues and cells associated with the disease [25,26]. It is generally reported that the oxidant-antioxidant balance is impaired in both AS patients and Behçet's patients. It has not been clarified how the oxidant/antioxidant mechanism is impaired in both diseases [18,19]. In our results, the deterioration of oxidant-antioxidant balance was found to be higher in AS patients than in BD patients. There is no study in the literature exactly explaining mechanism of oxidant/antioxidant system patients with BD and ankylosing spondylitis. However, in a AS mouse model was found to be had higher levels of TNF-α, IL-1β, and IL-6 pro-inflammatory cytokines, and lower levels of SOD, CAT, and GSH [27]. Whereas this study is not parallel to the current study in terms of SOD activity. Perhaps this oppositional relationship is not suitable for humans because we do not know which biomolecules play a role in this mechanism.

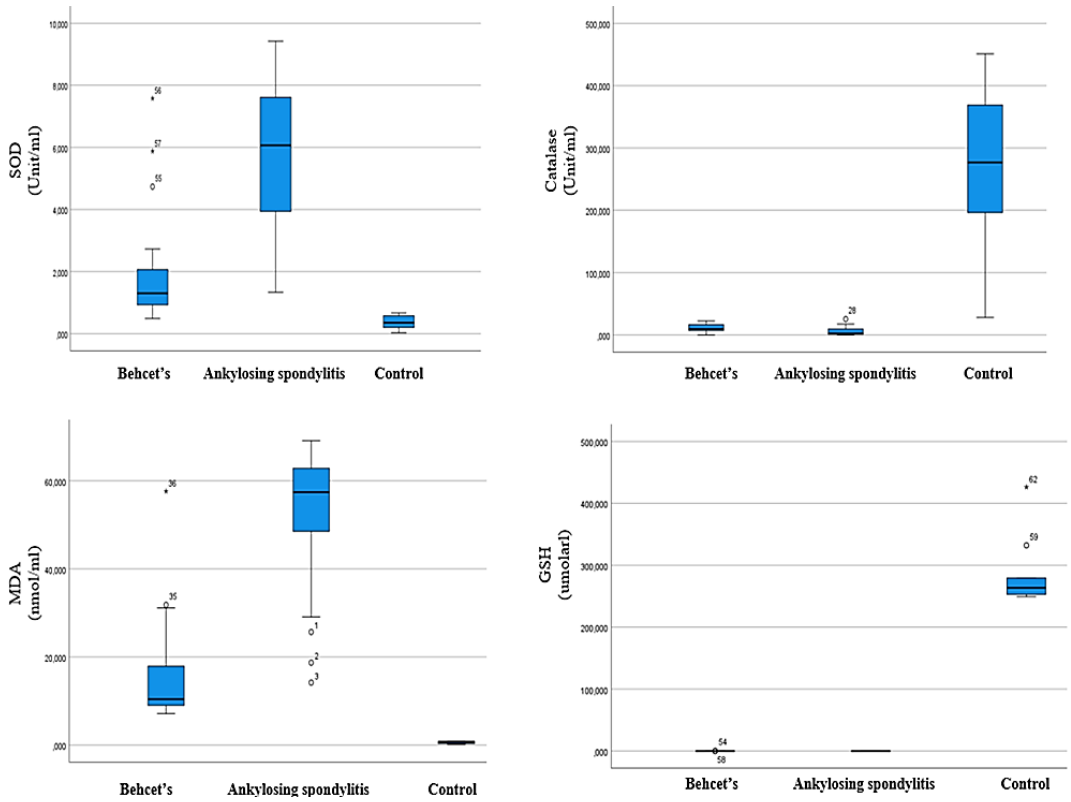

Fig. 1. Box plots of SOD, GSH, Catalase and MDA parameters. SOD: Superoxide dismutase, MDA: malondialdehyde and GSH: Reduced glutathione

Table 1. Superoxide dismutase, catalase, malondialdehyde, and reduced glutathione levels of both groups as well as patient characteristics

Variables	Ankylosing spondylitis, N=34	Behcet's disease, N=24	Control N=20	P value
Male, N (%)	22 (64.7)	15 (62.5)	10 (50)	0.86**
Age (Mean±SD)	46.34±10.07	38.50±11.03	42.55±13.01	0.21*
Erythrocyte, ($x10^6$/µL)	4.96±0.51	4.87±0.43	5.04±0.49	0.47§
Hemoglobin, (g/dl)	14.00±1.53	13.87±1.22	14.25±1.30	0.72§
HCT, (%)	41.36±4.06	41.05±3.17	42.02±4.1	0.76§
SOD (Unit/ml), Median (min-max)	6,07 (1,34-9,42)	1,30 (0,49-7,58)	0.30 (0.03-0.75)	0.001*b 0.001*a
Catalase (Unit/ml), Median (min-max)	2,38 (0,19-25,51)	9,44 (0,47-22,47)	303.45(28-510)	0.001*b 0.001*a
MDA (nmol/ml), Median (min-max)	57,44 (14,18-69,10)	10,43 (7,14-57,64)	0.72(0.16-0.92)	0.001*b 0.001*a
GSH (µmolar), Median (min-max)	0,029(0,003-0,044)	0,023(0,001-0,087)	272.8(230.1-426)	0.001*b 0.001*a

**P values were estimated with Mann-Whitney test. **P values were estimated with chi-square test. §P values were estimated with student t test. *bP values were calculated for Bechet's disease. *aP values were calculated for ankylosing disease.*

Until now, Behçet's disease was thought to be an autoinflammatory disease triggered by exogenous factors [28]. The results of the examination of familial BD suggest that genetic factors are effective in the pathogenesis of this disease [28]. In some studies, conducted in BD, it has been reported that HLA-Cx14, HLA-Cw15, HLA-B51, HLA-A28 and HLA-B12 alleles play an important role [29]. In addition, several studies have reported that TNF-α, GIMAP, IL-4, CCR1/CCR3 and TLR-4 genes are also associated with BD. In addition, it has been reported that microorganisms and heat shock proteins have a crucial role in the etiology of BD by activating the autoimmune system [28,29].

Similarly, ankylosing spondylitis is an immune-mediated chronic inflammatory disease. It has been reported that the immune system related biomolecules including HLA-B gene alleles (especially HLA-B27 and HLA-B40), IL-12B, IL-6R, helper T cells 17 and 23, endoplasmic reticulum aminopeptidase 1 and 2 (ERAP-1,2) are play a crucial role in its etiopathogenesis [29,30]. Although these reported mechanisms play a vital role in the pathogenesis of both diseases, oxidant/antioxidant status may also affect the pathogenesis or disease progression in patients with these autoinflammatory disorders [29,30].

The present study has several limitations. a) The study was conducted only with patients in and around Adana province, so it does not represent the entire Turkish population. b) The sample size of BD patients in the current study is limited.

4. CONCLUSION

In conclusion, considering the inconsistency of the results of the studies in the literature, we strongly recommend the inclusion of high-potential antioxidants that will strengthen the antioxidant defence mechanism and reduce peroxidation as supportive medical treatment according to SOD and catalase activities and GSH molecule levels in both patient groups.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that there are no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

CONSENT

All the patients or their legal guardians signed the informed consent.

ETHICAL APPROVAL

The present study was confirmed by the Ethics Committee of Çukurova University Faculty of Medicine in Türkiye.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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