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# Effects of oxidative stress, DNA damage, and inflammation in multiple sclerosis: A clinical perspective

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**Abstract**. Multiple sclerosis (MS) is a demyelinating nervous system disease known for its lesions and manifests itself with attacks. According to some theories, inflammation and oxidative stress play an important role in MS. With this study, we aimed to examine the levels of oxidative stress, inflammation and DNA damage in MS patients and to get an idea about the course of the disease from these data. The research comprised patients diagnosed with MS between the ages of 18 and 60. Photometric techniques were used to determine serum native thiol (NT), total thiol (TT), total antioxidant status (TAS), and total oxidant status (TOS) levels. The oxidative stress index (OSI), disulfide (DIS) level, and percentages of DIS/TT, DIS/NT, and NT/TT were determined with mathematical calculations. Inflammation biomarkers tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) were measured by photometric methods with commercially purchased ELISA kits. DNA damage was detected using alkaline single-cell gel electrophoresis. TOS, OSI, and DIS levels, as well as DIS/NT and DIS/TT percentages, IL-1β, IL-6, TNF- α and DNA damage levels were shown to be statistically significantly increased in MS patients than in the healthy control group ($p <$ 0,001), according to the study's findings. Furthermore, TAS, TT, and NT levels were decreased in MS patients. Inflammation occurs as a result of oxidative stress in MS patients and causes DNA damage. Our results show that clinicians should consider oxidative stress, inflammation, and DNA damage when evaluating MS's development.

***Keywords*:** central nervous system; multiple sclerosis; oxidative stress; reactive oxygen species; thiol/disulfide homeostasis.

## 1. Introduction

The most prevalent non-traumatic chronic nervous system disease affecting adults, multiple sclerosis (MS) causes attacks and damages the central nervous system (CNS) [1, 2]. With the socioeconomic effects of the condition, MS incidence is rising globally. Though the fundamental causes of MS and the processes causing this rise are still unknown, it is obvious that complex gene-environment interactions are crucial [3]. Low blood vitamin D levels, Epstein-Barr virus infection, and smoking is likely to contribute to the development of MS, according to the disease's epidemiology. People with MS may now be identified gradually earlier in the course of the disease because of advancements in diagnostic techniques and criteria [2, 3].

Multiple variables, including genetic, environmental, and many others, have a role in the etiology of MS [4]. Although the etiology is not completely known, it is thought to include T-cell-mediated inflammation for proteins associated with myelin as well as a potential function for B cells. Over the years, the McDonald criteria have been established and modified to help diagnose MS. Magnetic resonance imaging (MRI) of the spinal cord and brain served as the foundation for the clinical presentation [4, 5]. Patients with MS don't have any viral or bacterial antigens, which implies that an autoantigen is what's causing the disease. MS patients' tissue and blood can be found to contain antibodies that target the lipids, proteins, and carbohydrates of the CNS myelin sheath. Clinicians treating the disease should comprehend MS's intricate etiology and axon integrity's significance [2, 3, 5].

The existence of many cells depends on oxygen. It is also one of the most toxic and reactive substances ever discovered. Intracellular homeostasis is maintained by balancing the oxidation and reduction (redox) processes, often known as the "intracellular redox balance," to protect against the potentially damaging effects of oxygen. Extreme circumstances can result in "oxidative stress," which is extremely detrimental to the cell and may even cause it to die [1, 6, 7]. Reactive oxygen species (ROS) and their impact on the development of MS are still poorly understood, despite claims that they play a significant role in the disease. Clinical findings on oxidative stress indicators in MS patients are conflicting [7, 8]. Overproduction of ROS results in oxidative

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stress, which has been linked to demyelination and axonal damage in MS and animal models. However, if the CNS infection occurs, it can result in significant, irreversible neuronal damage [7-9]. Therefore, it is necessary to create novel treatment strategies that regulate and modulate inflammatory reactions within the CNS.

In this study, biomarkers for oxidative stress, DNA damage, and inflammation in MS patients were examined in relation to treatment, prognosis, or pathogenesis of the disease.

## 2. Experimental

Inclusion criteria for the study comprised patients who applied to the Neurology Outpatient Clinic at Basaksehir Cam and Sakura City Hospital in 2022 and were determined to have MS based on their Magnetic Resonance Images (MRI), Cerebrospinal Fluid (CSF) results, and physical activity findings. The study included 50 patients diagnosed with MS and 50 healthy volunteers, and the patients ranged in age from 18 to 60 years old. Sample collection was initiated following ethical consent from eligible patients. Approximately 3 mL of blood from the patients was transferred to both the Biochemistry gel tube and the EDTA tube. The blood in the gel tube was taken into the centrifuge tube and separated into serum at 3000 x g in 10 minutes. Serum samples that had been separated were put into Eppendorf tubes and kept at -80°C until analysis. The study was approved by the local ethics committee with the number 22/254.

### *2.1. Measurement of TAS, TOS, OSI*

The TAS and TOS levels of the samples were assessed using a photometric method with commercially available kits. The TAS and TOS values units were mmol Trolox Equivalent/L and mol $H_2O_2$ Equivalent/L, respectively. To generate the oxidative stress index, TOS/TAS was performed with the formula:

$$\text{OSI (arbitrary unit)} = (\text{TOS, } \mu\text{mol } H_2O_2 \text{ eq./L}) / (\text{TAS, } \mu\text{mol Trolox eq./L}) \times 10^{-1}$$

### *2.2. Measurement of native thiol (NT), total thiol (TT), and disulfide (DIS)*

The NT and TT levels in the samples were determined using a photometric method with commercially available kits (Rel Assay Diagnostics, Mega Tip, Turkey). By detecting half of the difference between the TT and NT groups, the number of dynamic disulfide bonds was calculated (DIS=(TT-NT)/2).

### *2.3. Measurement of inflammatory biomarkers*

Specific commercial ELISA kits were used to measure serum interleukin-1 beta, interleukin 6, and tumor necrosis factor-alpha concentrations according to the manufacturer's instructions (Bioassay Technology Laboratory, China). A spectrophotometric plate reader was used to specify concentrations at 450 nm (Varioskan Flash Multimode Reader, Thermo, Waltham, USA).

### *2.4. DNA damage assessment*

The previously described alkaline single-cell gel electrophoresis technique was used to analyze leukocyte DNA damage (Comet Assay) [10]. Briefly, a low melting temperature agarose was used with 6 µL of thawed whole blood (0.7%) (Sigma-Aldrich-A9414). It was then placed on slides covered with agarose gel (1%) (Sigma-Aldrich-A4718) after it reached the normal melting temperature, covered with a coverslip, and allowed to be set up in a cold environment. Coverslips were removed from the slide when the gel was completely set, and cells were then lysed by soaking the slides in a lysis solution for at least 4 hours. It was subsequently put through electrophoresis (300 mA) in an alkaline buffer for 20 minutes (pH = 13). Fluorescence microscopy (Excitation: 546 nm, Emission: 20 nm) was used to examine cells stained with ethidium bromide (5 mg/mL) (Sigma-Aldrich-E7637) after electrophoresis. Using the Comet Assay analysis software IV, 50 cells on average were counted to determine DNA tail percentages (Perceptive Instruments, Suffolk, UK).

### *2.5. Statistical analysis*

The data were analyzed using SPSS version 25.0. (IBM, Armonk, NY, USA). Numerical data (such as oxidative/antioxidant status parameters, TOS, TAS, and OSI) were given as mean standard deviations in contrast to categorical variables, which were provided as the number of patients (n). Mann-Whitney *U* and Chi-square tests were utilized appropriately to compare the control and patient groups. The difference between the groups was displayed with a 95% confidence interval. At $p < 0.05$, statistics were considered significant.

### *2.6. Limitations*

Patients with MS were selected regardless of the attack stage. This is considered a limitation of our study.

## 3. Results and discussion

The study involved a total of 100 individuals, 50 of whom were MS patients and 50 of whom were healthy volunteers. The MS patient group ranged in age from 18 to 60, and Table 1 displays the gender distribution of the disease as well as the duration after diagnosis.

**Table 1**. Demographic characteristics of MS patients and healthy control group

| | | **MS (n = 50)** | **Control (n = 50)** |
|---|---|---|---|
| **Age (year)** | | 46.10 ± 8.48 [¥] | 46.12 ± 10.48 [¥] |
| **Gender** | *Male* | 28 (%56) | 28 (%56) |
| | *Female* | 22 (%44) | 22 (%44) |
| **Diagnosis time (year)** | | 9.54 ± 4.50 [¥] | - |

[¥] Mean ± Standard deviation; MS: Multiple Sclerosis

MS is believed to be a central nervous system-related autoimmune disease in which the myelin sheath is damaged, resulting in axonal loss, manifesting its effects with attacks [11-14]. Since being able to predict diseases is important for clinicians, we aimed to clinically

examine oxidative stress and inflammation biomarkers and DNA damage in patients diagnosed with MS.

Oxidant markers in serum such as TOS (13.92 ± 1.75 vs. 10.62 ± 1.35 µmol $H_2O_2$/L, $p < 0.001$), TAS (0,84 ± 0.18 versus 1.12 ± 0.13 mmol Trolox Equiv./L, $p < 0.001$), and OSI (17.50 ± 6.11 vs. 9.62 ± 1.83 arbitrary units, $p < 0.001$) in MS patients and healthy controls are shown in Figure 1.

These findings showed that TAS levels were considerably decreased in MS patients compared to the healthy control group, whereas TOS and OSI levels were significantly increased ($p < 0.001$).

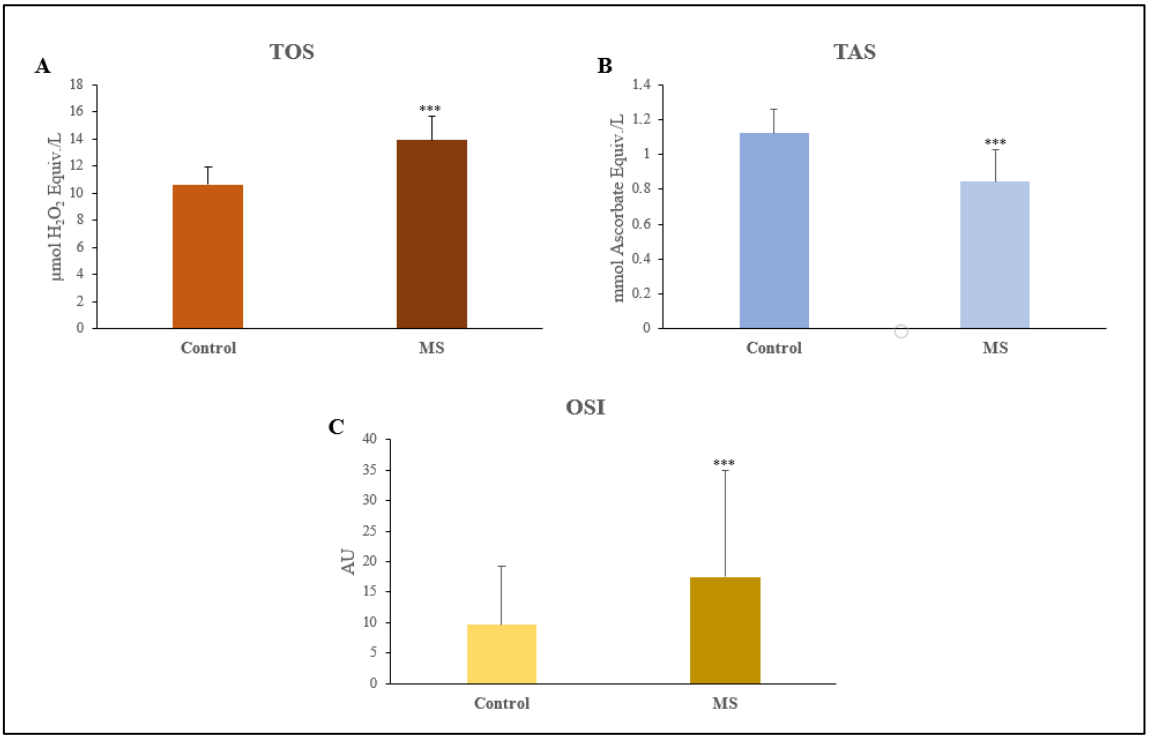


**Figure 1**. Oxidant markers in serum A) Total Oxidant Status, B) Total Antioxidant Status, and C) Oxidative Stress Index. MS: Multiple Sclerosis. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). $p < 0.05$ value was considered statistically significant. AU: Arbitrary Unit.

Oxidative stress is essential in multiple sclerosis and occurs before the inflammatory response during relapse. MS patients experience antioxidant deficiency and a general oxidative state [15]. The oxidative equilibrium in metabolism is reported to degrade with aging and cause major damages in the latter stages of MS [11, 16]. In the study of Oncel et al., it was stated that there was no difference between TAS, TOS, and OSI levels in both MS patients and the control group [17]. According to some studies, women are more susceptible to oxidative stress than males. Female MS patients had higher TOS and OSI levels and lower TAS levels, according to the study by Vasic et al. [18]. In previous studies, it is thought that thiol (-SH) groups are important in MS disease, and these parameters play a role in the development of MS [15, 19]. In our study, TOS and OSI levels were statistically significantly increased in MS patients compared to the control group, and TAS levels were significantly decreased.

Both endogenous and external factors may generate ROS. Activated inflammatory cells including macrophages, neutrophils, and eosinophils, as well as ROS produced by mitochondria and peroxisomes, are examples of endogenous sources. Xenobiotics are also metabolized by cytochrome P450 oxidoreductases. ROS are continuously produced in mitochondria as products of respiration (1–5% of oxygen consumed), and are generally known as the primary cause of oxidative damage in aerobic species [20]. Active immune cells create ROS throughout the disease, causing oxidative stress and thereby contributing to demyelination, axonal damage, and inflammation processes [16,21].

**Table 2**. Dynamic thiol/disulfide parameters in multiple sclerosis patients and healthy control group (n = 50)

| | **MS** | **Control** | ***p*-value** |
|---|---|---|---|
| NT *µM* | 263.67 ± 46.19¥ | 462.21 ± 49.56¥ | 0.001 |
| TT *µM* | 432.37 ± 59.75¥ | 616.74 ± 64.19¥ | 0.001 |
| DIS *µM* | 84.35 ± 41.52¥ | 77.26 ± 42.21¥ | 0.399 |
| DIS/NT % | 35.72 ± 25.22¥ | 17.58 ± 10.99¥ | 0.001 |
| DIS/TT % | 18.76 ± 7.62¥ | 12.11 ± 5.58¥ | 0.001 |
| NT / TT % | 62.48 ± 15.24¥ | 75.78 ± 11.15¥ | 0.001 |

¥ Represents the mean ± standard deviation; p < 0.05 value was considered statistically significant; MS: Multiple Sclerosis, NT: Native Thiol, TT: Total Thiol.

Vural et al. discovered that the mean disulfide/TT, disulfide/NT, and disulfide values of MS patients who had an attack were significantly higher [19]. According to our findings, while NT, TT, and NT/TT ratios were significantly lower in MS patients compared to controls, disulfide/NT and disulfide/TT levels were found to be high and significant (Table 2).

Only disulfide levels were found to be higher in MS patients compared to controls, but there was no statistical significance.

IL-1β, IL-6, and TNF-α levels in serum were examined, and the values are shown in Figure 2. According to the results, the levels of IL-1β, IL-6, and TNF-α were found to be statistically significantly increased in MS patients compared to the control group.

Inflammatory cytokines are important immune response regulators, and their overproduction may result in autoimmune diseases [22]. It is known that pro-inflammatory cytokines released from B cells, in which inflammation is seen in addition to axonal damage in MS, play an important role in the pathogenesis of MS [23, 24]. In our study, the levels of pro-inflammatory cytokines IL-6, IL-1β, and TNF-α were examined to observe inflammation. According to the results of our study, IL-6, IL-1β, and TNF-α levels were found to be statistically significant and higher in patients with MS compared to the control group. In the study of melatonin supplementation by Yosefard et al. [25], it is stated that there is no significant difference in TNF-α levels in patients with MS, but a significant decrease in IL-1β levels compared to the control group. It suggests that melatonin could be a therapeutic choice.

Blood was drawn into EDTA tubes and tested for leukocytes and DNA damage. The findings are given in Figure 3. These findings showed that oxidative stress-induced DNA damage levels were statistically significantly increased in MS patients than in the healthy control group.

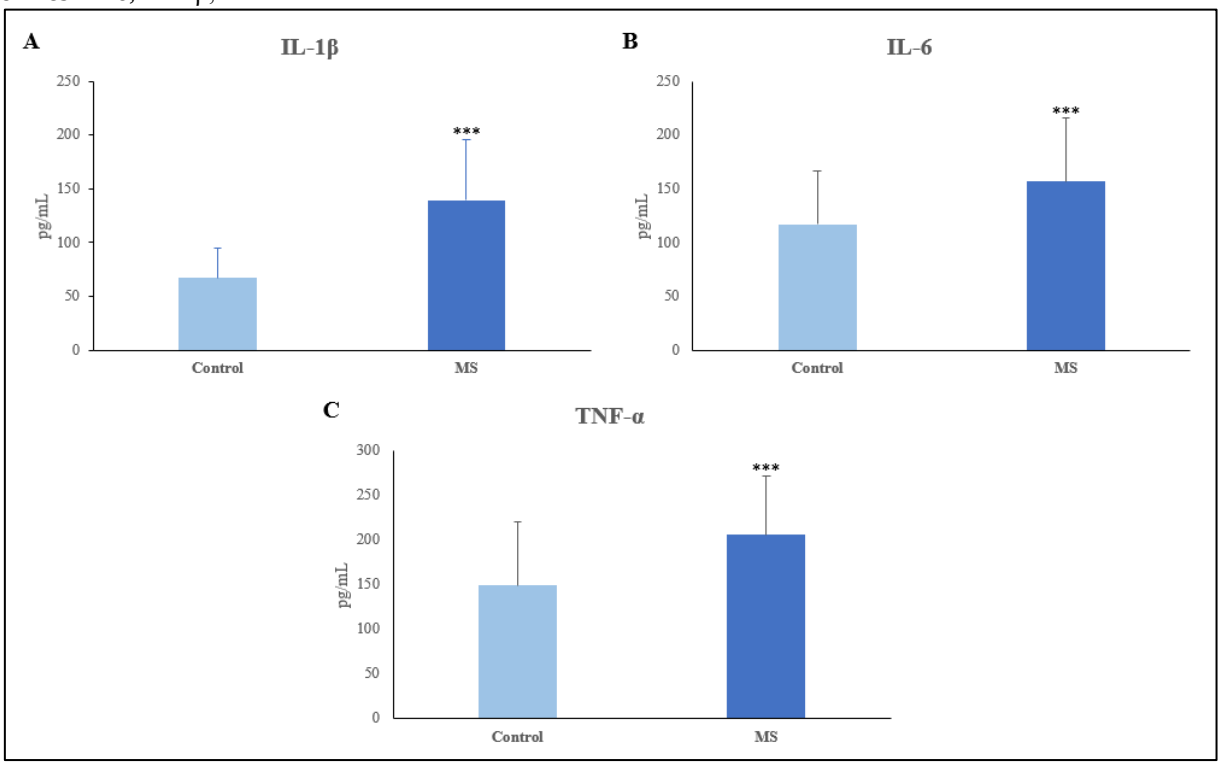


**Figure 2**. Serum inflammation biomarkers A) Interleukin-1beta, B) Interleukin-6, and C) Tumor necrosis factor-alpha. MS: Multiple Sclerosis. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). $p < 0.05$ value was considered statistically significant.

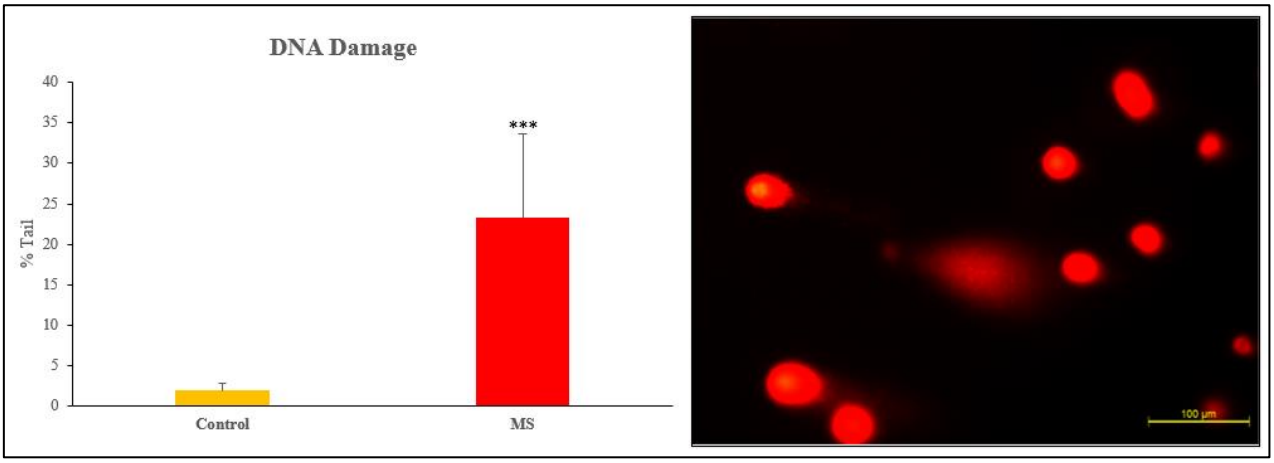


**Figure 3**. DNA damage levels of patients with Multiple Sclerosis (MS) and the control group. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). $p < 0.05$ value was considered statistically significant.

DNA damage can be caused by various chemical, physical and environmental factors, as well as molecules generated by cellular metabolism. There is evidence that an excess of ROS, also known as free radicals, is one of

the underlying causes of many disorders, including autoimmune disorders, by causing DNA damage [26-29]. In the study of Menezes et al., higher levels of DNA damage were detected in MS patients compared to the control group [30]. According to our data, the DNA damage levels of MS patients were statistically higher than the control group. With the accumulation of ROS, oxidative stress occurred, and DNA damage was induced in MS patients.

As a result, excessive production of ROS in the body has created an environment for oxidative stress in patients with MS. Oxidative stress triggers inflammation and increases levels of pro-inflammatory cytokines. Inflammation and ROS caused DNA damage in patients with MS. Although these results are compatible with the literature, new studies are needed.

## 4. Conclusions

Neurodegenerative diseases are challenging to diagnose and difficult to control. The treatment of MS becomes increasingly challenging as the disease progresses, and more research needs to be done for this. Therefore, it is crucial for clinicians to predict diseases and make an early diagnosis. Our results show that clinicians should consider oxidative stress, inflammation, and DNA damage when evaluating MS's development.

## Conflict of interest

Authors declare no conflict of interest.

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