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Original Article

Evaluation of Oxidative System Parameters in Alzheimer's Disease Before Medical Treatment

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SUMMARY

Background: Alzheimer's disease (AD) is the most common reason for dementia and is one of the most important causes of morbidity and mortality in the aging population. A crucial component of AD is the brain's sensitivity to oxidative stress. We aimed to determine the oxidative load of patients with AD who had just been diagnosed and had not yet begun medical treatment.

Methods: To assess oxidative load before drug administration, we compared the levels of serum total antioxidant (TAS), oxidant status (TOS), paraoxonase (PON1), arylesterase (ARES), total thiol (THIOL) levels in patients just diagnosed with AD (n = 41) and control (n = 45) with the totally 86 individuals. AD and control groups oxidative stress index (OSI) ratio was calculated too.

Results: There was a statistically significant difference between the AD and control groups for mean TAS, TOS, and OSI levels with a 95% confidence level ($p^{\text{TAS}} = 0.001$, $p^{\text{TOS}} = 0.005$, $p^{\text{OSI}} = 0.001$). There was not a statistically significant difference between the groups in terms of mean PON1, ARES, and THIOL values. Significantly negative and positive correlations were found for the interested parameters in both groups.

Conclusion: The increase in antioxidative capacity in patients with AD may be related to ARES supported by TAS, and THIOL levels suggest, that those protein oxidation mechanisms are effective in the progress of AD disease before medication.

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1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia. The main reason for AD is the loss of nerve cells and synapses in various parts of the central nervous system. It is a kind of neurodegenerative disease that progresses progressively characterized by a decrease in cognitive functions, and self-care deficiencies, with various neuropsychiatric and behavioral disorders.¹ Recent studies showed that, while the incidence of AD was 5/1000 in the 65–70 age, it was observed that this frequency increased to 70/1000 in 85 years of age and above period.² The incidence and prevalence of AD increase dramatically every year. It has been reported that the number of Alzheimer's patients is around 35 million worldwide. It is estimated that this number will rise to over 100 million in 2050.³ In Turkey, the prevalence of AD was found to be 11% in individuals over 70 years of age.⁴

The clinical diagnosis of AD is based on clinical evaluation, use of neuropsychiatric tests and diagnostic criteria, as well as the exclusion of other bases of dementia. However, the definitive diagnosis is only possible with a neuropathological examination in the post-mortem period.⁵ In the aging population, AD is one of the leading

reasons for morbidity and mortality, since it is the primary cause of dementia. Currently, the progression of the disease can be slowed down with the current treatments, so early diagnosis is crucial. For this reason, possible biomarkers for the early diagnosis of AD and exclusion of non-Alzheimer's dementia have received increasing attention.⁶

Neuronal degeneration in AD has not yet been clarified, although oxidative stress/antioxidant balance has been thought to be an important reason for the pathogenesis of AD.⁷ The accumulation of free radicals and oxidative stress contributes to AD pathology. This leads to oxidative load, peroxidation of lipids, and neuronal degeneration in the brain.⁸

The main reason of the oxidative stress is the imbalance between oxidative and anti-oxidative mechanisms and changes in total antioxidative stress (TAS) and total oxidative stress (TOS) levels. Hence, the accumulation of reactive oxygen species (ROS) arises in all living organisms. The structure of proteins, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA) is affected by the activity of ROS.^{9,10} These activities lead to structural deformations of biological components, which may result in the losses of functions in several tissues.^{11–13} ROS are derived primarily from enzymatic and nonenzymatic processes. Several enzymes are involved in the production of ROS, including those involved in respiration, prostaglandin synthesis, phagocytosis, and cytochrome P450.¹⁴ Free radicals can also

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be produced nonenzymatically, i.e., when oxygen reacts with organic compounds or when cells are exposed to ionizing radiation.¹⁵ Exogenous and endogenous free radicals both contribute to free radical formation. Free radicals are created by a multitude of factors including inflammation, ischemia, infection, cancer, excessive exercise, and mental stress. A variety of external influences can produce free radicals, including pollution, heavy metals, certain chemicals, tobacco smoke, alcohol, and radiation. The body then degrades or metabolizes these exogenous compounds, and free radicals are formed as by products.^{16–19}

AD is a pathologically complicated disease, including oxidative stress, cell-cycle changes, with many other biochemical changes.²⁰ It has been shown that there is a link between paraoxonase (PON1) activity and the progress of AD.²¹ Human PON1 plays a role in arylesterase (ARES) activity. It can hydrolyze organophosphate compounds and is associated with high-density lipoprotein. It is thought that the PON1 polymorphism can be related to the development of neurological diseases.²² The role of PON1 status and oxidative stress in many neurodegenerative diseases has been shown by several studies.^{21–23}

Dynamic thiol-disulfide homeostasis plays an important role in antioxidant protection by leading to the up-regulation of antioxidant capacity.^{7,24} It has been identified that many diseases are caused by oxidative stress in which dynamic thiol-disulfide homeostasis in the organism is affected.⁷ Some other findings suggested that the -108 polymorphism shows the greatest effect on ARES activity.²¹

Although oxidative stress parameters have been increasingly studied in many disorders, there is no study has evaluated such parameters as; TAS, TOS, PON1, ARES, and total thiol (THIOL) in patients with AD before medical treatment. Therefore, it is aimed to evaluate what kind of oxidative-antioxidative balance exists in the progression of AD before the drug load.

2. Materials and methods

2.1. Participants

We conducted this study on 86 subjects who were treated in the Home Health Unit, Kütahya Health Sciences University, Kütahya, Turkey. Forty-one unrelated Alzheimer's patients (19 males, 22 females) were included in the patient group and 45 (20 males, 25 females) healthy subjects (age-matched) were included in the control group. Both the control and patient groups were chosen among the Turkish population. The diagnosis of AD was established based on the criteria proposed by the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) and the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV).^{25,26} Power analysis was performed by calculating the statistical power for AD and control group (n = 45) using two-tailed test and 80% power of confidence interval with alpha = 5% level of significance.

All procedures were explained to individually all subjects and written informed consent was obtained. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki as reflected in a prior approval by the Institution's Human Research Committee. The study was approved by the Clinical Research Ethics Committee of Kutahya Health Sciences University.

2.2. Enzyme-Linked Immunosorbent Assay (ELISA) Analyses and Oxidative Stress Index calculation

Peripheral blood samples (5 mL) were obtained from each sub-

ject by venipuncture. After collecting the whole blood from each participant into a blood tube without any agent, we left the tubes at room temperature for approximately 20–30 min to allow the blood to clot. In order to isolate the fibrinogen precipitate, we centrifuged the clot at 3000 rpm for 15 min, resulting at serum. Then, all serum samples were stored at Eppendorf. After centrifugation, the serum of everyone was stored at -80 °C until Enzyme-Linked Immunosorbent Assay (ELISA) analysis.

Serum concentrations of TAS (Rel Assay Diagnostics, Turkey, REF No: RL0017, LOT No: JE 14042A), TOS (Rel Assay Diagnostics, Turkey, REF No: RL0024, LOT No: JE 14048Og), PON1 (Rel Assay Diagnostics, Turkey, REF No: RL0031, LOT No: JE14028P), ARES (Rel Assay Diagnostics, Turkey, REF No: RL0055, LOT No: JR13017AR), and THIOL (Rel Assay Diagnostics, Turkey, REF No: RL0178, LOT No: AL 13011TL) were analyzed by ELISA kits. The oxidative stress index (OSI) was calculated according to the following formula: OSI (arbitrary unit) = TOS (μmol H₂O₂ Equiv./L)/TAS (μmol Trolox Equiv./L) × 100.^{27,28}

2.3. Statistical analyses

Statistical analyses were performed by SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) 16.0 package program. Power analysis was performed by calculating the statistical power by two-tailed test for study. The power of the study was 80%, the effect size was 0.55, the interval alpha = 5% level of significance and the total sample size was 84 (AD group n = 42, Control group n = 42). The conformity of the data to the normal distribution was evaluated with the Kolmogorov Smirnov test ($p < 0.05$) and non-parametric tests were used accordingly. Serum levels of interest parameters were given as mean ± standard error of the mean (SEM). Serum levels of relevant parameters were given as mean ± standard deviation. The data obtained were evaluated using the Mann-Whitney U test, t-test, and Spearman's correlation tests. All p -values < 0.05 were accepted as statistically significant.

3. Results

Mean ages were found as 75.97 ± 6.43 for the Alzheimer's group and 78.35 ± 5.45 for the control group. The serum levels of PON1, ARES, and THIOL were found as 174.78 ± 166.70 U/L, 602.67 ± 207.20 U/L, and 1209.37 ± 762.31 μmol/L in the Alzheimer's group. The serum levels of PON1, ARES, and THIOL were found as 178.92 ± 140.46 U/L, 585.83 ± 168.90 U/L, and 1297.13 ± 556.69 μmol/L in the control group. No statistically significant differences were found for these parameters between the two groups ($p^{\text{PON1}} = 0.335$, $p^{\text{ARES}} = 0.489$, $p^{\text{THIOL}} = 0.125$) (Table 1).

The serum levels of TAS, TOS, and OSI were found as 12.7 ± 0.29 μmol/L, 5.838 ± 22.40 μmol/L and 0.459 ± 0.23 arbitrary unit in the Alzheimer group, and 9.6 ± 0.48 μmol/L, 8.185 ± 23.37 μmol/L and 0.852 ± 0.65 arbitrary unit in the control group. There were statistically significant differences in these parameters between the groups ($p^{\text{TAS}} = 0.001$, $p^{\text{TOS}} = 0.005$, $p^{\text{OSI}} = 0.001$) (Figure 1).

Table 1
Serum levels of PON1, ARES and THIOL in control and Alzheimer groups.

	Alzheimer (n = 41)	Control (n = 45)	p -values
PON1 (U/L)	174.78 ± 166.70	178.92 ± 140.46	0.584
ARES (U/L)	602.67 ± 207.20	585.83 ± 168.90	0.754
THIOL (μmol/L)	1209.37 ± 762.31	1297.13 ± 556.69	0.644

Values were compared with Mann Whitney U test. Data are expressed as mean ± SD. $p > 0.05$ vs. control group.

ARES, arylesterase; PON1, paraoxonase; THIOL, total thiol.

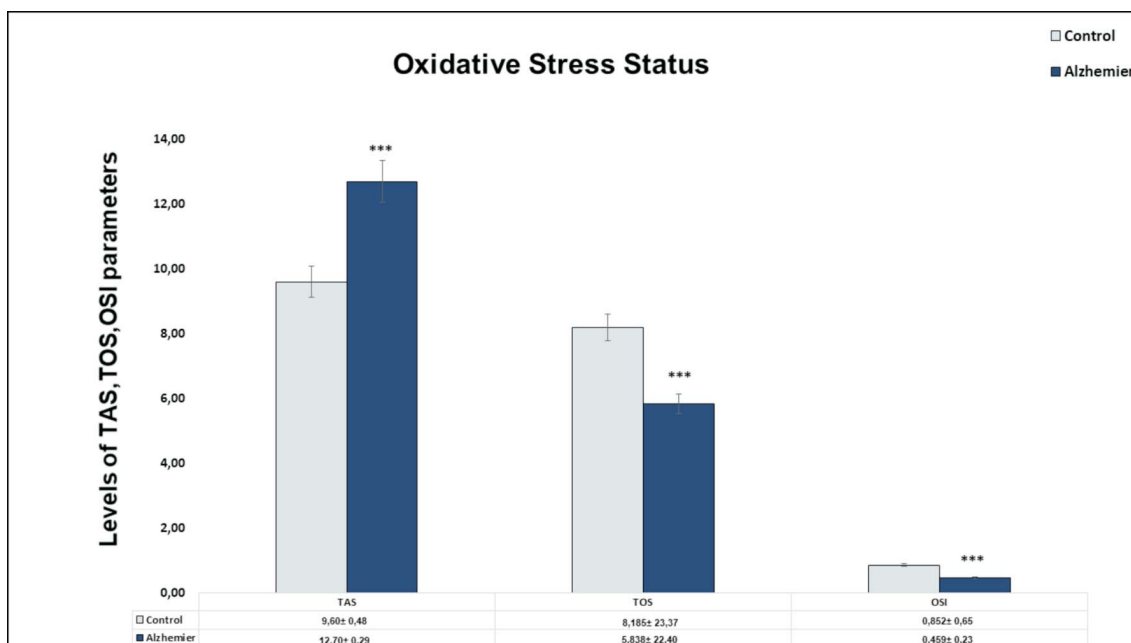


Figure 1. Serum levels of total antioxidant status (TAS), total oxidative status (TOS) and oxidative stress status (OSI) in control and Alzheimer groups. Data are mean ± SD. ** $p < 0.01$ vs. control group (Mann-Whitney U test), *** $p < 0.005$ vs. control group (Mann-Whitney U test).

There were significant negative correlations between TAS versus TOS, OSI, and THIOL ($p^{TAS-TOS} = 0.045$, $p^{TAS-OSI} = 0.000$, $p^{TAS-THIOL} = 0.007$) a significant positive correlation between OSI versus TOS and THIOL parameters ($p^{OSI-TOS} = 0.000$, $p^{OSI-THIOL} = 0.016$), THIOL versus PON1, and ARES parameters ($p^{THIOL-PON1} = 0.001$, $p^{THIOL-ARES} = 0.000$) in controls (Table 2).

There was a significant negative correlation between TAS versus TOS and OSI ($p^{TAS-TOS} = 0.000$, $p^{TAS-OSI} = 0.000$), a significant positive correlation between TOS versus OSI and THIOL parameters ($p^{TOS-OSI} = 0.000$, $p^{TOS-THIOL} = 0.018$), THIOL versus OSI, PON1, ARES parameters ($p^{THIOL-OSI} = 0.018$, $p^{THIOL-PON1} = 0.005$, $p^{THIOL-ARES} = 0.039$), PON1 versus ARES ($p^{PON1-ARES} = 0.000$) in AD (Table 2).

4. Discussion

An unbalanced status for oxidative stress plays an important role in several kinds of disease by affecting mainly functional loss of tissues. The brain is the most sensitive tissue of these tissues that sense oxidative damage because of its oxygen consumption.^{7,9,10}

The importance of oxidative stress and many biochemical processes in the pathophysiology of AD is important.²⁰ Early diagnosis of AD is important for the early treatment of this disease. Therefore, to distinguish AD from other dementia in the early diagnosis of this disease, it is necessary to determine the specific biomarkers.²⁹ In our study, the difference between the serum TAS, TOS, PON1, ARES, and THIOL levels of the newly diagnosed and untreated Alzheimer's patients and control groups were determined by considering the relationship between oxidative stress and Alzheimer's disease.

If the increase in oxidative capacity and decrease in antioxidative capacity occurs, this causes many diseases via oxidative damage.³⁰ The total oxidant and antioxidant status values can be used as a general parameter that can detect all free radicals likewise synthesized and the acting antioxidants.³¹ The oxidative imbalance, which occurs probably secondary to other biochemical processes has been accepted as an early indication of AD progress specifically than other neurodegenerative diseases.³² In literature plasma levels of TAS in AD have been found to decrease.^{31,33} Additionally, Guidi et al. could not find any differences in oxidative status for patients with

Table 2
Serum levels of TAS, TOS and OSI, PON1, ARES and THIOL in control and Alzheimer groups.

	TAS (mmol/L)	p	TOS (μmol/L)	p	OSI (arbitrary unit)	p	ARES (U/L)	p	THIOL (μmol/L)	p
Control										
TAS	.	.	-0.315	0.045*	-0.709	0.000***	.	.	-0.415	0.007**
TOS	0.849	0.000***
OSI	0.374	0.016*
PON1	0.672	0.000***	0.514	0.001***
ARES	0.539	0.000***
Alzheimer										
TAS	.	.	-0.758	0.000***	-0.897	0.000***
TOS	0.94	0.000***	.	.	0.351	0.018*
OSI	0.353	0.018*
PON1	0.633	0.000***	0.413	0.005**
ARES	0.309	0.039*

Values were correlated with the Spearman's correlations tests. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ is statistically significant positively correlation between control and AD patient's data.

ARES, arylesterase; OSI, oxidative stress status; PON1, paraoxonase; TAS, total antioxidant status; THIOL, total thiol; TOS, total oxidative status.

AD.³¹ Controversially to the literature, we have found a statistically significant decrease for OSI because of a significant increase in TAS and a decrease in TOS level. Additionally, all expected correlations were found to be statistically significant between OSI and TAS and TOS in both groups. We believe that this result is related to our population and the new diagnoses of patients with AD. Additionally, we think that the compensatory physiological mechanisms of the body against oxidative stress developing in the early stages of AD disease may be effective. Therefore, if we want to determine AD disease with biochemical markers in the early stage, we foresee it is necessary to explain the oxidative and antioxidative mechanisms in detail.

Studies have shown a relationship between low PON1 activity and high oxidative stress in several diseases such as cardiovascular disease, type 2 diabetes, and neurodegenerative diseases.^{34–37} Variation in the PON1 gene frequencies can change the PON1 activity 40 times in different populations. Therefore, in Alzheimer's patients, it is important to determine the relationship between genetic polymorphism and the state of the PON1 gene.³⁸ Some studies have indicated no difference in PON1 enzyme activity between Alzheimer's patients and control groups in different populations.^{39,40} In Helbecque et al.'s study, it is unclear how PON1 affects the risk of AD.⁴¹ We have shown that PON1 activity is lower in Alzheimer's patients compared to controls although this decrease is not statistically significant. Additionally, we could not observe a significant correlation between OSI and PON1. Therefore, we think that PON1 activity in the physiological compensatory antioxidative mechanisms for the early stage of AD has not played an effective role.

ARES has an antioxidative effect like PON1. In the literature, it is suggested that low ARES activity can be used as a preliminary diagnostic criterion for AD.²¹ Contrary to what the literature suggests, we have found a high ARES activity in the Alzheimer's group, although it is not significant. We believe that ARES activity is responsible for the higher antioxidant capacity in the Alzheimer's group. These results show that in the developmental stage of AD, different pathways play a role in oxidative-antioxidative balance which needs to be clarified. In this respect, the fact that other oxidative parameters could not be measured at different developmental levels of AD constitutes the limitation of our study.

It is known that oxidative stress-related diseases can affect the dynamic thiol-disulfide homeostasis in the organism.^{42,43} Considering that oxidative stress is effective in the development of AD, dynamic thiol/disulfide homeostasis is thought to worsen in patients with AD. Therefore, the level of dynamic thiol-disulfide in body fluids is considered a new marker for the correct definition of oxidative stress status in explaining AD pathogenesis. In literature, they have found that total thiol and native thiol levels of patients with AD were lower than the control group.⁷ Following the literature, we have also found a low serum level of total thiol in AD compared to control.

5. Conclusion

Serum TAS level was found to be statistically higher in the AD group compared to the control group. However, serum TOS levels and OSI values were found to be statistically lower in the AD group. According to our results, we think that the increase in antioxidative capacity in Alzheimer's patients is related to ARES. A statistically insignificant increase in ARES in the AD group indicates different pathways in the effectiveness of antioxidative mechanisms in patients with AD.

As a result, unlike the literature, the increase in antioxidative

capacity in the AD group is an indication that there may be a difference between populations. Simultaneously, the retrieved data suggest that there are differences in the regulation of oxidative-antioxidative balance in different stages of AD development.

Compliance with ethical standards

The study protocol conforms to the ethical guidelines of the Declaration of Helsinki as reflected in a prior approval by the institution's human research committee.

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Conflict of interest

On behalf of all authors, the corresponding author states that there are no conflicts of interest.

Ethical approval

The study was approved by the Clinical Research Ethics Committee of Kutahya Health Sciences University (2018/14-12).

Informed consent

All procedures were explained to individually all subjects and written informed consent was obtained.

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