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Malondialdehyde, Superoxide Dismutase, Melatonin, Iron, Copper, and Zinc Blood Concentrations in Patients with Alzheimer Disease: Cross-sectional Study

Ramazan Ozcankaya, Namik Delibas[1]

Departments of Psychiatry and [1]Biochemistry and Clinical Biochemistry, University of Suleyman Demirel School of Medicine, Isparta, Turkey

Aim. To investigate the oxidative stress hypothesis in patients with Alzheimer type dementia.

Method. Serum melatonin, Zn, Cu, Fe, and malondialdehyde (MDA) concentrations and erythrocyte superoxide dismutase (SOD) activity were measured in patients with Alzheimer disease. Mini-Mental State Examination (MMSE) scores were obtained for the patients and their age- and sex-matched healthy controls.

Results. MMSE score was 16.8 ± 1.3 in patients with Alzheimer disease, and 28.2 ± 2.4 in control group. Melatonin levels were lower in the Alzheimer disease group (170.5 ± 5.2 pg/mL) than in the control group (205.2 ± 5.8 pg/mL) ($p < 0.001$). Fe, SOD, and MDA levels were higher in Alzheimer disease group (131.7 ± 4.8 μg/dL, 1,510 ± 90 U/g Hb, and 38.1 ± 4.7 nmol/mL; respectively) than in the control group (97.1 ± 4.1 μg/dL, 1,120 ± 50 U/g Hb, and 17.2 ± 1.6 nmol/mL; respectively) ($p < 0.001$ for all comparisons). A statistically significant negative correlation between melatonin and SOD ($r = -0.421$, $p = 0.014$) and a positive correlation between age and Fe ($r = 0.325$, $p = 0.049$) were found only in the Alzheimer disease group. Correlation between age and melatonin was positive ($r = 0.481$, $p = 0.006$) in Alzheimer disease group and negative ($r = -0.472$, $p = 0.009$) in the control group.

Conclusion. Melatonin blood concentration was significantly decreased, and Fe and MDA levels were increased in the patients with Alzheimer disease. We believe that low level of melatonin, especially if there is a simultaneous increase in Fe level, is associated with the development of Alzheimer disease.

Key words: *Alzheimer disease; antioxidants; iron; malondialdehyde; melatonin; oxidative stress; superoxide dismutase*

There are many etiologic hypotheses for Alzheimer disease, such as genetic defect (1), slow or latent virus disorder (2), energy metabolism defect (3), altered processing of amyloid precursor protein (4), deficiency of neurotrophic factors (5), glutamate toxicity (6), mitochondrial defect (7), trace element neurotoxicity (8), and free radical-induced neuron degeneration in the oxidative stress hypothesis (9). It is possible that several of these hypotheses, e.g., trace element neurotoxicity, excitotoxicity, mitochondrial defect, and oxidative stress, may interact as pathogenetic mechanisms in Alzheimer disease (10). Central nervous system is especially vulnerable to free radical damage due to brain's high oxygen consumption rate, its abundant lipid content, and the relative paucity of antioxidant enzymes compared with other tissues (11).

The neurotoxic trace element hypothesis in Alzheimer disease is relevant to the oxidative stress hypothesis. Elements receiving the most attention in Alzheimer disease are aluminum (Al) (12), mercury (Hg) (13), and iron (Fe) (9). Of these, iron may have the most important pathophysiologic role as a catalyst for free radical generation by virtue of having a loosely bound electron and the ability to exist in more than one valence (9). The stable redox state of iron is Fe^{+3}, but it is the bivalent form, Fe^{+2}, that is capable of transferring one electron and facilitating free radical generation. Several studies have indicated a disruption of iron metabolism in the brain of patients with Alzheimer disease (10). Increased iron level in the cortex of patients with Alzheimer disease was documented by histochemical techniques (14). Instrumental neutron activation analysis of bulk neocortical specimens obtained from patients with Alzheimer disease revealed large but statistically insignificant increase in iron level compared with the controls, especially in the gray matter (9). In another study, significant iron increase was found in the amygdala, hippocampus, and olfactory pathway of patients with Alzheimer disease compared with their age-matched controls (15).

Studies of antioxidant enzymes in Alzheimer disease have not shown a consistent pattern. Superoxide dismutase concentration was elevated in all brain regions of the patients with Alzheimer disease, without reaching statistical significance (10). Several studies found no significant alterations in either Cu-Zn or Mn-superoxide dismutase activities in the brain

(16,17), whereas Richardson (18) reported 25-35% reduction of superoxide dismutase in frontal cortex, hippocampus, and cerebellum of patients with Alzheimer disease. Another report described elevated superoxide dismutase activity in the caudate nucleus in patients with Alzheimer disease (19).

The exact role of the free radical scavenging effects of melatonin in aging has not been clarified. Melatonin is a direct free radical scavenger and indirect antioxidant, and its reduction with age may be a factor for increased oxidative damage in the elderly (20). Melatonin could also play a role in aging and age-related diseases, probably related to its efficient antioxidant activity (21). The potential clinical benefit of melatonin may be its use in the treatment of many pathophysiological conditions, including a variety of neurodegenerative diseases, such as Alzheimer's (20), probably because melatonin has the ability to prophylactically reduce oxidative damage (21). These studies suggest that melatonin may have beneficial effects in some patients with Alzheimer disease. Gonca et al (22) suggested that increased levels of lipid peroxidation products may play a role in aging, and exogenous melatonin may delay the aging process of tissues by means of its free radical scavenging effects.

The aim of this study was to test the hypothesis of decreased activity of defense system protecting tissues from free radical damage in patients with Alzheimer disease by measuring the level of malondialdehyde (an end-product of lipid peroxidation), antioxidants, including superoxide dismutase and melatonin, and metals (Fe, Cu, and Zn) associated with the free radical production in erythrocyte and serum samples of patients with Alzheimer disease.

Subjects and Methods

Subjects

Seventy-four residents of Isparta Resting Home were recruited consecutively after systematic physical and psychiatric examination. Patients for the study had to be older than 60 years and had to fulfill the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) criteria for Alzheimer disease (23). Patients who fulfilled one or more of the following six criteria were excluded from the study: 1) patients whose life expectancy appeared to be less than 3 months because of life threatening diseases; 2) patients taking steroids; 3) patients whose cognitive functions could not be assessed because of blindness or deafness; 4) patients taking medications, such as iron for anemia; 5) illiterate patients; and 6) patients with medical disorder other than dementia (Fig. 1). Written informed consent was obtained either from the patient or from authorized person at the resting home after the study procedure was explained. The patients with the diagnosis of Alzheimer disease according to DSM-IV criteria were also assessed with the Mini Mental State Examination (MMSE) (24).

Inclusion criteria for controls were the following: age over 60 and MMSE score above 26 (25). Illiterate persons, persons on medications, and those having any other medical and psychiatric disorder were excluded from the study. There were 39 patients and 35 controls included in the study. Twelve patients (5 with psychosis, 3 with mental retardation, 3 who refused to participate, and one receiving medication) and 10 controls (6 under 60 years of age, 2 who refused to participate, and 2 receiving medication) who had not met inclusion criteria were excluded from the study. The final patient group consisted of 27 patients with Alzheimer disease, and the control group comprised 25 healthy subjects (Fig. 1). The mean age of the patient group, which consisted of 19 men and 8 women, was 72.3 ± 6.5 years (range 62-79, median 68). The mean age of the control group, which included 16 men and 9 women, was 64.4 ± 7.2 years (range 61-71, median 65). The groups did not significantly differ in age ($t = 1.3$, d.f. = 50, $p = 0.088$) and sex (chi-square = 1.83, d.f. = 1, $p = 0.064$), but they differed in MMSE scores ($t = 21.51$, d.f. = 50, $p < 0.001$), (Table 1).

Assays

All venous blood samples taken between 7 and 8 a.m. after 12 h of fasting were collected in polystyrene tubes and vacutainers containing heparin. The tubes were centrifuged at 500 G for 15 min. Erythrocyte pellets were obtained immediately from the heparinized blood by centrifugation at 3,000 G. Plasma and buffy coat were then removed and the erythrocytes were washed three times in 5 mL cold 0.9% NaCl solution. Erythrocytes were hemolyzed by adding cold distilled water. Samples (serum and erythrocyte hemolyzate) were stored at -20ºC until analysis.

Serum melatonin concentrations were measured twice using the Melatonin I 125 RIA kit (DDV Diagnostica, Marburg, Germany).

Erythrocyte superoxide dismutase activity was measured by the method of Flohe and Ötting (26). The reduction rate of cytochrome c by superoxide radicals was monitored at 550 nm by use of the xanthine-xanthine oxidase system as superoxide radicals source. Superoxide dismutase competed for superoxide and decreased the reduction rate of cytochrome c. One unit of superoxide dismutase was defined as the amount of enzyme causing 50% inhibition in the rate of cytochrome c reduction. Superoxide dismutase activity was expressed in units per gram hemoglobin (U/g Hb).

Serum Fe, Cu, and Zn concentrations were measured using the Hitachi Z-8000 polarized Zeeman Atomic Absorption Spectrophotometer (Hitachi Ltd, Tokyo, Japan).

Serum malondialdehyde levels were measured by the double heating method of Draper and Hadley (27). The principle of the method was based on the spectrophotometric measurement of the color occurred during the reaction to thiobarbituric acid with malondialdehyde. Concentration of thiobarbituric acid reactive substances (TBARS) was calculated by the absorbance coefficient of malondialdehyde-thiobarbituric acid complex 1.56×10^5 $cm^{-1}M^{-1}$ and expressed in nmol/mg protein.

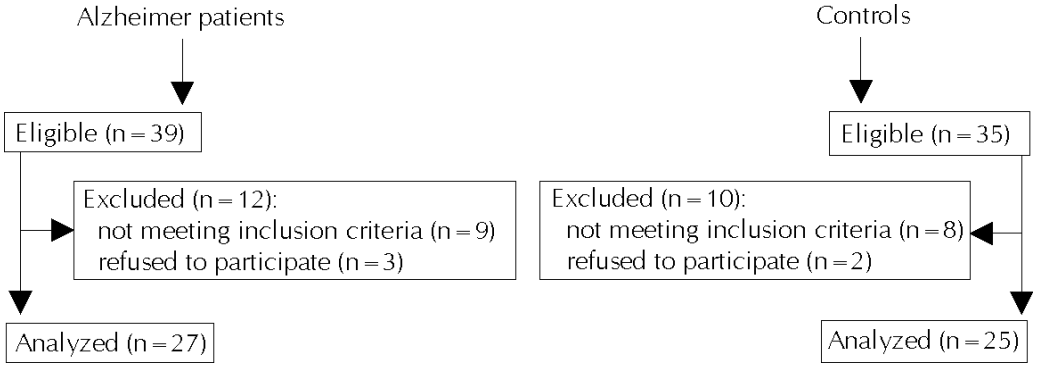

Figure 1. The design of the study.

Statistical Analysis

Statistical analysis was performed with SPSS software package (Version 9.0 for Windows). Data were expressed as mean ± standard deviation (SD). For comparison of two groups of continuous variables, independent samples t-test was used. Chi-square test was used for comparison of gender characteristic in two groups. Correlations between variables were assessed with Pearson's correlation coefficients (r). A probability value of $p < 0.05$ (two-tailed) indicated a statistically significant difference.

Results

Erythrocyte superoxide dismutase activity, levels of serum malondialdehyde, melatonin, Zn, Cu, and Fe, and MMSE scores are given in Table 1. Melatonin levels were significantly lower in the patient group than in the control group ($t = 15.0$, d.f. = 50, $p < 0.001$). No statistically significant difference in serum Zn and Cu levels was found between the groups ($t = 1.78$, d.f. = 50, $p = 0.075$; and $t = 0.65$, d.f. = 50, $p = 0.515$, respectively), but serum Fe levels were significantly higher in patient group ($t = 27.86$, d.f. = 50, $p = < 0.001$). In comparison with controls, the group of patients with Alzheimer disease had significantly higher erythrocyte superoxide dismutase activity ($t = 9.89$, d.f. = 50, $p < 0.001$) and higher serum malondialdehyde concentration ($t = 8.07$, d.f. = 50, $p < 0.001$).

Table 1. Findings in patients with Alzheimer disease and healthy control group

Parameter	Alzheimer disease (n = 27)	p	Control (n = 25)
MMSE[a]	16.8 ± 1.3	< 0.001	28.2 ± 2.4
Melatonin (pg/mL)	170.5 ± 5.2	< 0.001	205.2 ± 5.8
Zn (μg/dL)	69.5 ± 2.0	0.075	67.8 ± 2.1
Cu (μg/dL)	76.1 ± 1.3	0.515	77.0 ± 1.5
Fe (μg/dL)	131.7 ± 4.8	< 0.001	97.1 ± 4.1
SOD[b] (U/gHb)	1510 ± 90	< 0.001	1120 ± 50
MDA[c] (nmol/mL)	38.1 ± 4.7	< 0.001	17.2 ± 1.6

[a]MMSE – Mini Mental State Examination.
[b]SOD – superoxide dismutase.
[c]MDA – malondialdehyde.

In the patient group, a positive correlation was found between age and superoxide dismutase ($r = 0.465$, $p = 0.05$), age and malondialdehyde ($r = 0.610$, $p = 0.001$), and age and Fe ($r = 0.325$, $p = 0.049$) (Fig. 2). The correlation between melatonin and malondialdehyde was negative ($r = -0.332$, $p = 0.045$) (Fig. 3), as well as the correlation between melatonin and superoxide dismutase level ($r = -0.421$, $p = 0.014$) (Fig. 4). Superoxide dismutase level showed a positive correlation with malondialdehyde level ($r = 0.470$, $p = 0.008$) in the group of patients.

In the control group, we found negative correlation between malondialdehyde and melatonin ($r = -0.395$, $p = 0.025$) and a positive correlation between age and superoxide dismutase ($r = 0.481$, $p = 0.038$), age and malondialdehyde ($r = 0.541$, $p = 0.019$), and superoxide dismutase and malondialdehyde ($r = 0.428$, $p = 0.045$). Correlation between age and melatonin level was negative in the control group ($r = -0.472$, $p = 0.0099$ (Fig. 5), but positive in the patient group ($r = 0.481$, $p = 0.006$) (Fig. 6). No significant correlation was found between other parameters.

Discussion

Melatonin level was lower and activity of erythrocyte superoxide dismutase, serum levels of malondialdehyde, and Fe were increased in patients with Alzheimer disease. We found positive correla-

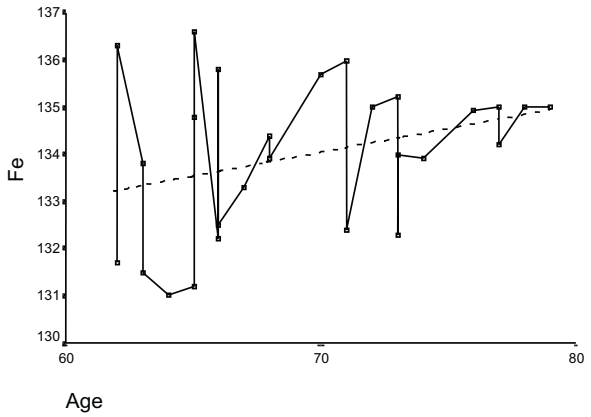

Figure 2. Correlation between age and Fe level in patients with Alzheimer disease ($r = 0.325$, $p = 0.049$). Full line – observed data; dashed line – estimation curve.

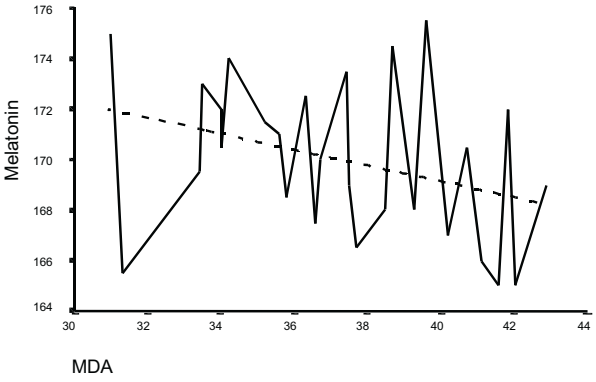

Figure 3. Correlation between melatonin and malondialdehyde (MDA) concentrations in patients with Alzheimer disease ($r = -0.332$, $p = 0.045$). Full line – observed data, dashed line – estimation curve.

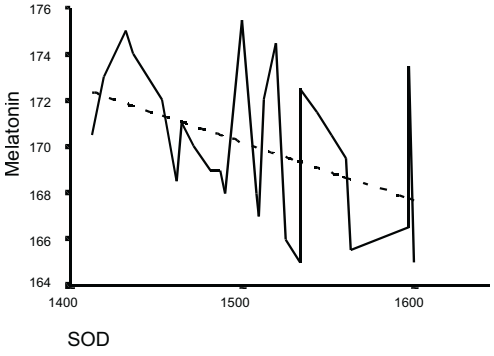

Figure 4. Correlation between melatonin concentration and superoxide dismutase (SOD) activity in patients with Alzheimer disease ($r = -0.421$, $p = 0.014$). Full line – observed data, dashed line – estimation curve.

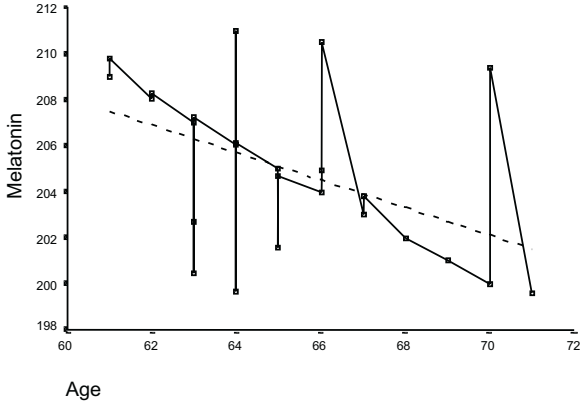

Figure 5. Correlation between age and melatonin concentration in control group (r=-0.472, p=0.009). Full line – observed data, dashed line – estimation curve.

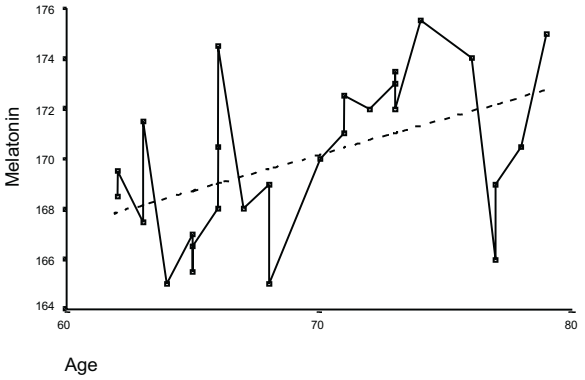

Figure 6. Correlation between age and melatonin concentration in patients with Alzheimer disease (r=0.481, p=0.006). Full line – observed data, dashed line – estimation curve.

tion between age and melatonin in patients with Alzheimer disease, but not in the controls. Additionally, we found positive correlation between Fe and age and negative correlation between melatonin and superoxide dismutase in patients with Alzheimer disease. These findings suggest that increased lipid peroxidation in Alzheimer disease group may be caused by increased free radical production and/or decreased antioxidant defense. We suspect that increased lipid peroxidation and decreased melatonin levels in patients with Alzheimer disease may be responsible for the risk relation between age and Alzheimer disease. Negative correlation between melatonin and superoxide dismutase and high Fe concentration in patients with Alzheimer disease may also be an important factor in the development of Alzheimer disease. This could imply that the administration of melatonin and Fe chelators may be therapeutically beneficial.

In an experimental study (22), plasma malondialdehyde levels in old rats were significantly higher than those in the young ones. Plasma malondialdehyde levels were lower in the group of old rats treated with melatonin, then in the control group. The results of our study show that one of the main reasons for high malondialdehyde (lipid peroxidation) in old patients could be melatonin deficiency. This means that decrease in melatonin seems to be related to aging. However, low melatonin levels in our patient group and statistically significant differences between controls and patients in our study could be explained not only by a decrease in melatonin due to aging, but also by a decrease in melatonin due to Alzheimer, which is probably much larger.

It is interesting that a correlation between melatonin and age was negative in the control group but positive in the patient group. At first it seems contradictory, but possible explanation could be that the increase in melatonin is compensatory to the increase in malondialdehyde and the free radical activity. However, this increase is insufficient because Alzheimer patients have lower melatonin levels than their age-matched controls. There are many possible interpretations of this correlation in patients with Alzheimer disease because of the complex ethiopathogenesis of the disease. Gonca et al (22) suggested that increased levels of lipid peroxidation products might play a role in aging and that exogenous melatonin might delay the aging process of tissues because of its free radical scavenging effects. The fact that some variables affecting cognitive status were not controlled, e.g., the onset and duration of the disease vs education and age, could be a limitation to our study, but the results were in accordance with previous research (20-22). However, more important limitation is that the circadian rhythm of melatonin was not considered in this study. We tried to overcome this handicap by taking blood samples from all patients at the same time.

High Fe levels can cause lipid peroxidation (15). Since patients with Alzheimer disease in our study had increased Fe levels, our results strongly support this view. Fe plays a very important pathophysiological role in free radical production (10). As Fe is a transition metal, it is associated with free radical production at high Fe levels. In the presence of H_2O_2, Fe may cause hydroxyl radical production by Fenton reaction. These results show that interventions to remove iron from the body may contribute to the treatment of Alzheimer disease.

Several studies have indicated a disruption of Fe metabolism in the cortex of subjects with Alzheimer disease (9,15). Significantly higher level of Fe was found in the amygdala, hippocampus, and olfactory pathway in patients with Alzheimer disease than in their age-matched controls (15). Our results were in accordance with these studies. The absence of a significant relation between age and Fe level in the control group, as opposed to the patient group, can be seen as a remarkable determinant.

According to our results and the results of other studies, increased malondialdehyde level is a result of aging. However, it seems that malondialdehyde increase alone is not enough for the development of

Alzheimer disease. Decreased melatonin level in patients with Alzheimer disease might reflect decreased antioxidant system, which might be correlated with the increased malondialdehyde level. The use of melatonin in the treatment of patients with Alzheimer disease could prevent neuronal degeneration by preventing lipid peroxidation.

Negative correlation between superoxide dismutase and melatonin observed in the patient group, points to the disrupted balance between oxidant and antioxidant system. Indeed, several reports have indicated increased activity of superoxide dismutase in patients with Alzheimer disease (19,28).

We conclude that lipid peroxidation increases due to both aging and the Alzheimer disease. Antioxidant defense system in normal aging is able to compensate for the peroxidation effects. However, in patients with Alzheimer disease, this compensation mechanism may become insufficient.

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Correspondence to:

Namik Delibas

Tip Fakültesi Hastanesi Klinik Biyokimya Laboratuvari

32260 Isparta, Turkey

ndelibas@hotmail.com