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# Plasma 8-isoPGF2α and serum melatonin levels in patients with minimal cognitive impairment and Alzheimer disease

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**Background/aim:** F2α-isoprostane is accepted as an oxidative stress indicator and melatonin shows neuroprotective effects by antioxidative and antiamyloidogenic influences. By measuring these in patients diagnosed with minimal cognitive impairment (MCI) and Alzheimer-type dementia, we intended to demonstrate whether the measurement of these markers contributes to early diagnosis of Alzheimer disease (AD) in the MCI stage or not.

**Materials and methods:** Three groups (n = 63) were created: the AD group, MCI group, and control group. Serum melatonin levels were measured by radioimmunoassay method, and plasma total 8-isoPGF2α levels were measured by enzyme immunoassay method.

**Results:** Significant differences were observed in the melatonin levels between the MCI group and AD group (P = 0.009), and in 8-isoPGF2α levels between the AD group and control group (P = 0.022). A negative correlation between mini-mental state examination (MMSE) scores and 8-isoPGF2α levels (r = –0.459, P < 0.001) and positive correlation between MMSE scores and melatonin levels (r = 0.317, P = 0.011) were found.

**Conclusion:** Although the plasma 8-isoPGF2α and serum melatonin levels in MCI were not found to be good early diagnostic markers to indicate risk of AD, results were found to support the role of oxidative stress in AD.

**Key words:** Alzheimer disease, minimal cognitive impairment, oxidative stress, isoprostane, melatonin

## 1. Introduction

Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by cognitive impairment. Before AD diagnosis, there is a long period with neuropathological changes (1–3).

Minimal cognitive impairment (MCI) is the clinical status of nondemented individuals who have memory deficits confirmed by objective tests. These memory deficits should not impair entire cognitive function or daily living activities (2). Annual conversion rate of MCI to dementia is reported as 10%–15% in different studies (4). The degenerative neuropathology of AD in the brain starts 20–30 years earlier than the clinical onset (5). Thus, MCI state is an important stage to determine and treat in people who are at risk of developing dementia.

Oxidative damage is present in early AD and also in MCI, and the role of oxidative stress is suggested in the etiopathogenesis of AD. When the balance between oxidant and antioxidant status shifts to the oxidant side, oxidative stress manifests itself in the form of protein oxidation, DNA oxidation, and lipid peroxidation (6). Due to high polyunsaturated fatty acid content of brain, which is sensitive to oxidative stress, primarily lipid peroxidation occurs in the brain (7–9).

Isoprostanes, prostaglandin isomers, are considered to be the most accurate indicators of oxidative stress (10). In vitro and in vivo measurements of isoprostane are sensitive and specific for lipid peroxidation (11). Isoprostane $F2_{\alpha}$-III and 8-isoprostaglandin $F2_{\alpha}$ are major products formed by free radical-catalyzed peroxidation of esterified arachidonic acid in membrane phospholipids (12).

The other parameter focused on is melatonin, which is suggested as an important direct free radical scavenger and indirect antioxidant (13–16). Melatonin is shown to be significantly effective at reducing oxidative damage in experimental models of the brain (15). In addition to reduced melatonin secretion during aging, more prominent decreases are reported in dementia (13,17). Daytime

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and nighttime melatonin levels are found to be lower in those aged 60 and above than in younger individuals, and patients with AD are also shown to have lower melatonin levels than individuals without the disease (13,18).

In this study we analyzed the plasma 8-isoPGF2$_\alpha$ levels and serum melatonin levels in nondemented control individuals and in patients diagnosed with MCI and Alzheimer-type dementia in order to demonstrate whether the measurement of these markers contributes to early diagnosis in the MCI stage or not.

## 2. Materials and methods

The study was conducted with the cooperation of the Biochemistry and Psychiatry Departments of the Süleyman Demirel University Medicine Faculty (Isparta, Turkey). The study protocol was approved by the Süleyman Demirel University Board of Ethics.

### 2.1. Subjects

Subjects older than 60 years of age (n = 63) from among residents of Isparta Atabey Rest Home, admissions to the Psychiatry Department, and field research were recruited to the study consecutively after a systematic physical and psychiatric examination by a psychiatry doctor.

For the assessment of cognitive status, a geriatric depression scale (19), global deterioration scale (20), activities of daily life scale (21), clock drawing test (22), behavioral pathology in AD scale (BEHAVE-AD) (23), and mini-mental state examination (MMSE) (24,25) were used. Patients whose life expectancy appeared to be less than 3 months because of life-threatening diseases, those taking steroids or vitamins, those whose cognitive functions could not be assessed because of blindness or deafness, and those with neurological or psychiatric disorders other than dementia were excluded.

In the study, three groups were created: the AD group (n = 20), MCI group (n = 21), and control group (n = 22). The diagnosis of MCI was established according to the Peterson criteria (26) and the diagnosis of dementia of the Alzheimer type was established according to DSM-IV criteria (22). The patients whose MMSE score was 24 or lower were accepted as having impaired cognitive function. The patients whose MMSE scores were higher than 26, who had normal cognitive functions, and who did not meet DSM-IV and Peterson diagnostic criteria were included in the control group. The AD group consisted of 20 patients (8 women and 12 men) with mean age of 79.05 ± 8.93 years and MMSE scores of 14.7 ± 4.3. The MCI group consisted of 21 patients (12 women and 9 men) with mean age of 73.95 ± 7.19 years and MMSE scores of 22.0 ± 1.3. The control group consisted of 22 individuals (9 women and 13 men) with mean age of 71.59 ± 6.65 years and MMSE scores of 27.9 ± 1.3.

### 2.2. Sample collection and preparation

After overnight fasting, between 0800 and 0900 hours venous blood samples were drawn into EDTA-containing tubes and polystyrene tubes. After centrifugation at 3000 × *g* for 10 min, plasma and serum samples were separated. Butylated hydroxytoluene (Sigma, Germany) was added to plasma samples, which were stored for isoprostane study (0.5% of plasma volume). Serum and plasma samples were stored and frozen at –80 °C until assayed.

### 2.3. Measurement of plasma total 8-isoPGF2$_\alpha$ levels

Plasma total 8-isoPGF2$_\alpha$ levels were measured using the Direct 8-iso-Prostoglandin F2$_\alpha$ Enzyme Immunoassay Kit, catalog number 900-091 (Correlate, Assay Designs, USA).

### 2.4. Measurement of serum melatonin levels

Serum melatonin concentrations were measured using the Melatonin Direct Radioimmunoassay (Serum/Plasma) Kit, REF RE29301 (IBL, Germany).

### 2.5. Statistical analysis

Statistical analysis was performed with SPSS 11.0 for Windows. For three-group comparison, ANOVA and ANCOVA tests were used, and for pairwise comparisons the post hoc Bonferroni test was used. Chi-square test was used for comparison of sex characteristics and Kruskal–Wallis ANOVA was used for comparison of age characteristics in the groups. Correlations between variables were assessed with Pearson's correlation coefficients (r) and Mann–Whitney U tests. Data are expressed as mean ± standard deviation (SD). $P < 0.05$ was accepted as significant.

## 3. Results

Melatonin levels were significantly higher in the MCI group than in the AD group ($P = 0.009$). The 8-isoPGF2$_\alpha$ levels were significantly higher in the AD group than in the control group ($P = 0.022$). A negative correlation between MMSE scores and 8-isoPGF2$_\alpha$ levels ($r = –0.459$, $p < 0.001$) and a positive correlation between MMSE scores and melatonin levels was found ($r = 0.317$, $P = 0.011$). Data were analyzed using ANCOVA and effect of age on melatonin was not significant ($F = 1.59$, $P = 0.960$). The results are shown in the Table.

## 4. Discussion

In several studies, cerebrospinal fluid (CSF) isoprostanes in patients with postmortem definitive diagnosis of AD and also plasma and urinary isoprostanes in living probable AD patients were measured. Elevated isoprostanes in the brain and CSF obtained during autopsy of patients diagnosed with AD have been reported (27,28). However, postmortem studies could not define whether the high isoprostane levels in CSF represent an early step in the neurodegeneration process as a result of oxidative stress by

**Table.** Age and 8-isoPGF2$_\alpha$ and melatonin levels in study groups.

| | Control group | MCI group | AD group |
|---|---|---|---|
| Age | 71.59 ± 6.65 | 73.95 ± 7.19 | 79.05 ± 8.93[a] |
| 8-isoPGF2$_\alpha$ (pg/mL) | 2014.9 ± 809.1 | 2580.7 ± 1239.7 | 3254.8 ± 2066.1[b] |
| Melatonin (pg/mL) | 11.88 ± 2.35 | 12.73 ± 5.57 | 9.02 ± 2.91[c*] |

Data are given as mean ± SD.
a: Significant difference when compared to control group (P = 0.006).
b: Significant difference when compared to control group (P = 0.022).
c: Significant difference when compared to MCI group (P = 0.009).
*: Effect of age on melatonin was not significant (ANCOVA, F = 1.59, P = 0.96).

lipid peroxidation or the final event. An increase in plasma or urine F2-isoprostanes in patients with AD or MCI has also been reported (29–31). However, in the literature there are conflicting findings in CSF, peripheral plasma, and urine F2-isoprostane levels in living individuals with clinically diagnosed AD (32,33). In addition, it was suggested in a recent study that the CSF levels of F2-isoprostanes and beta-amyloid 1-42 are associated with the severity of neuropsychological symptoms, and also that the median levels of F2-isoprostane and F4-neuroprostanes were significantly higher in the cognitive impairment group than the control group (34).

In our study, a statistically negative correlation between MMSE scores and plasma

8-isoPGF2$_\alpha$ levels suggests that an increase in oxidative stress might be associated with cognitive decline. Plasma 8-isoPGF2$_\alpha$ levels of both the AD and MCI groups were higher than in the control group, but statistical significance was found only between the AD group and control group. The MCI group diagnosed clinically needs to be determined precisely. Patient selection criteria, assay method type, and the effect of additional diseases in elderly patients can alter the results of studies.

In addition to its major role in the regulation of circadian rhythms, the antioxidative and neuroprotective roles of melatonin in preventing the accumulation of amyloid have been demonstrated. In AD patients and elderly people, low melatonin values and impaired melatonin rhythm have also been shown. Before appearance of clinical symptoms, the changes in melatonin may be an early event in the development of AD (13,15,35). However, no association was found between postmortem CSF melatonin levels and the onset, duration, and severity of disease (17). Ventricular CSF melatonin levels were determined in patients with postmortem neuropathological staging and it was suggested that the decrease in CSF melatonin levels might be an early event in the development of AD (36). Wu et al. investigated the correlation between CSF and pineal melatonin content and plasma-CSF melatonin levels and suggested melatonin as an early marker in AD due to decreased melatonin levels since the earliest stage of AD (37). On the other hand, recent clinical studies suggested melatonin use for the preventive treatment of major neurodegenerative disorders (38). Wade et al. investigated the addition of prolonged-release melatonin to conventional anti-AD therapy (acetylcholinesterase inhibitor with or without memantine) and determined cognitive function improvement in mild to moderate AD cases (39).

In our study, both the control group and MCI group mean serum melatonin levels were higher than in the AD group. However, a significant difference was only found between the MCI and AD groups; the differences between the AD and control and the MCI and control groups were not significant. Existence of a positive correlation between MMSE score and levels of melatonin shows a protective effect in terms of cognitive status. Absence of a significant difference between the AD and control group, slightly higher mean melatonin levels in the MCI group compared to the control group, and the coincidence of values in the data from the three groups suggest that serum melatonin levels are not predictive in the MCI stage.

In our study, the levels of melatonin were measured only in the morning. In several studies, nocturnal melatonin levels in AD have shown selective decrease (40,41). However, during the day, increased melatonin levels were also shown. In melatonin production, in addition to the changes with age, time changes in the rhythm of melatonin are reported (37,42). Pineal gland size and melatonin secretion of individuals has also been determined genetically (13). Perhaps especially in MCI individuals, longitudinal studies showing changes in melatonin rhythm may be more meaningful.

As with many peripheral markers investigated for early diagnosis of AD, our findings are limited due to other tissue interactions and lack of specificity to the brain.

In conclusion, although the plasma 8-isoPGF2$_\alpha$ and serum melatonin levels in MCI were not found to be good early diagnostic markers to show risk of AD, in combination with other diagnostic markers these parameters may be supportive. This study also supports the suggestions about the role of oxidative stress in AD.

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