INTRODUCTION

Alzheimer’s disease (AD) is a progressive disorder that manifests itself with characteristic pathological changes in the brain, such as cognitive dysfunction, memory loss, senile plaques, and neurofibrillary tangles. Although a clinical diagnosis of AD may be made after other causes of dementia have been excluded, a definite diagnosis may only be possible following postmortem evaluation of brain tissue for typical neuropathological findings. With the aim of early initiation of treatment, the role of several biomarkers for the definite antemortem diagnosis of AD has been evaluated in recent years. The diagnostic criteria for AD were revised in 2007 and medial temporal lobe atrophy as a magnetic resonance imaging finding, as well as the novel cerebrospinal fluid (CSF) biomarkers, were considered supportive of diagnosis. To date, three CSF biomarkers have been described: beta-amyloid 1-42 (Aβ1-42), total tau (t-tau), and phospho-tau-181 (p-tau). However, obtaining a CSF sample may be challenging, which highlights the need for the determination of biomarkers from peripheral blood.

Apelin is a neuropeptide that was first segregated from bovine stomach tissue in 1998. It is an endogenous ligand for the APJ receptor. The human preproapelin gene is located on chromosome Xq25-26.1. The apelin preproproteins consist of 77 amino acid residues that are cleaved into biologically active C-terminal fragments of various sizes. The apelin peptides, including 13 (65-77), 17 (61-77), and 36 (42-77) amino acids, are all capable of binding to APJ.
Among apelin isoforms, apelin-13 has the highest plasma concentration and plays the most significant role in neuroprotection. The apelin/APJ system has several important functions in the body, such as blood pressure regulation, cardiac contractility, immunity, glucose metabolism, water homeostasis, cell proliferation, angiogenesis, and neuroprotection.\(^6\) Besides its neuroendocrine functions, some studies have shown that apelin has a protective effect against N-methyl-D-aspartic acid (NMDA) mediated excitotoxicity in hippocampal neurons. Excitotoxicity is the pathological process by which neurons are damaged and killed by the overactivations of receptors for the excitatory neurotransmitter glutamate, such as the NMDA receptor and AMPA receptor. Apelin-13 also possibly suppresses phosphatidylinositol-3 kinase (PI3K) related apoptosis in human osteoblasts.\(^7\) Recent studies have also demonstrated protective effects of apelin-13 against cardiomyocyte injury due to ischemia. Neuronal cell death, brought about by necrosis and apoptosis, is a frequently encountered characteristic feature of acute and chronic neurodegenerative disorders.\(^8\) Many studies have reported a concentration-dependent neuroprotective effect of apelin-13 against serum deprivation (SD)-related apoptosis where cortical neurons are subjected to apoptosis via both caspase-dependent and caspase-independent pathways. Increased reactive oxygen species (ROS) production, mitochondrial depolarization, cytochrome C production, and caspase activation all play an important role in SD-induced neuronal apoptosis.\(^9\) Furthermore, PI3K inhibitions, as well as activation of extracellular signal-regulated mitogen-activated protein kinase (MAPK), have both been linked with SD-related neuronal apoptosis.\(^10\)

Under normal conditions, there is equilibrium between cellular prooxidants and antioxidants. But the presence of certain environmental factors or stressors causes an increase in the production of ROS resulting in an imbalance in favor of prooxidants. This results in the oxidative stress phenomenon.\(^11\) ROS are produced continuously by the mitochondrial electron transport chain and soon cleared by endogenous antioxidant systems.\(^12\) Oxidative stress and neuronal damage develop after levels of ROS exceed the cellular antioxidant capacity, or when antioxidant systems are insufficient.\(^13\) ROS may interact with several biomolecules like DNA, RNA, proteins, fats, and carbohydrates causing oxidative injury.\(^11\) Oxidative modification of biomolecules has been shown to result in cellular dysfunction.\(^14\) Increased ROS production is the main mechanism behind SD-induced apoptosis and neuronal death. It has been postulated that the anti-ROS activity of apelin is one of its many protective mechanisms.\(^15\)

2 | METHODS

This study was undertaken in the outpatient clinic of the department of neurology with the approval of the local ethics committee. A total of 31 patients (13 male patients and 18 female patients) with AD and 30 healthy controls (9 male controls and 21 female controls) were enrolled in the study. Patients were newly diagnosed with AD and took no therapy yet. The control group consisted of age- and gender-matched healthy individuals. All participants were subjected to a detailed medical history and careful physical examination and those with signs or symptoms of advanced heart failure, chronic obstructive lung disease, a systemic inflammatory disorder, a malignant condition, or a history of smoking were excluded from the study. The cognitive status of all participants was evaluated using the Mini-Mental State Examination (MMSE).\(^16\) Participants with a score of ≤25 were diagnosed as having dementia according to Diagnostic and Statistical Manual IV criteria. These patients were evaluated according to workgroup National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) protocol for a diagnosis of possible AD.\(^2\) Healthy control groups were also evaluated with MMSE and were proven to have cognitive scores in the safe range.

Blood samples were obtained from all participants by venipuncture of the antecubital vein between 8.00 AM and 9.00 AM following an 8 to 12 hour overnight fast. Samples were transferred to plastic vacuum gel tubes, which were then centrifuged for 10 minutes at 1452 g. Sera were stored in Eppendorf tubes at ~80°C.

Serum levels of apelin-13 were measured using Bachem Human apelin-13 (Cat. No. S-1416) enzyme-linked immunosorbent assay (ELISA) kits on a SEAC RADIM Company ALISEI analyzer, with a reference range of 0 to 100 ng/mL. TAS was measured by an automatic method as described by Erel et al, which quantifies the body’s TAS against potent free radicals (Rel Assay Diagnostic). Trolox equivalent/L units were used. Measurements of TOS were also done using an automated colorimetric method described by Erel et al (Rel Assay Diagnostic). Results were given as micromolar H\(_2\)O\(_2\) equivalent/L. Oxidative stress index (OSI) was calculated by dividing TOS values by TAS values (OSI [arbitrary unit] = TOS [mmol H\(_2\)O\(_2\) equivalent/L]/TAS [mmol Trolox equivalent/L]).

2.1 | Statistical analysis

All statistical analyses were performed using the Number Cruncher Statistical System (NCSS) 2007 and Power Analysis and Sample Size (PASS) 2008 statistics software (Utah). Values for numerical variables were provided as mean ± standard deviation or median (minimum-maximum) depending on normality of distribution whereas values of categorical variables were provided as frequency/rate. Group comparisons were done with Student t test or Mann-Whitney U test and correlation analyses by using Pearson’s correlation coefficient. A P value of less than 0.05 was considered statistically significant.

3 | RESULTS

The mean ages of patients and controls were 72.73 ± 6.17 and 75.54 ± 5.27 years, respectively. Two groups were age and
gender-matched. As per study protocol, patients in the AD group had significantly lower MMSE scores than their healthy counterparts.

Patients had significantly lower serum levels of apelin-13 compared with healthy controls. The mean TAS value was also significantly lower in the patients’ group. TOS and OSI values of patients were higher compared with controls but it was not statistically significant (Table 1).

Apelin-13 had a moderate positive correlation with TAS values ($r = 0.346; P > 0.05$). A moderate, albeit statistically insignificant negative correlation, was observed between TOS and TAS ($r = −0.328; P > 0.05$). Interestingly, MMSE levels showed significant moderate correlations with both TAS and TOS values. No correlation could be established between other parameters (Table 2).

### 4 | DISCUSSION

Research in recent years has focused on establishing the pathophysiological mechanisms that are involved in the development of AD, with special attention given to histopathological changes, such as widespread neuronal death, amyloid plaque formation, and neurofibrillary tangles. The presence of early indicators of tangle formation in certain areas of the brain, such as the entorhinal cortex, is more pronounced in the clinical diagnosis of AD. Although amyloid β peptide (Aβ), which is the main constituent of amyloid plaques, is known to be toxic to neurons, accumulation of Aβ facilitated by APP expression itself in transgenic mice did not result in a sufficient degree of neuronal death, suggesting that other factors may also be involved in disease progressions, such as impaired bioenergetics, oxidative stress, and inflammation. Excessive ROS production beyond cellular antioxidant capacity results in oxidative injury. Although the brain comprises only 2% of total body weight, it consumes 20% of total body oxygen, which explains why brain cells are inherently more sensitive to oxidative injury than other cells.

Defects in antioxidant defense systems gradually result in the production of oxidatively damaged cellular macromolecules. Unhindered oxidative overload leads to lipid peroxidation, protein oxidation, and other indicators of oxidative stress, which have been linked with several neurodegenerative disorders, including AD, Parkinson’s disease, amyotrophic lateral sclerosis, tardive dyskinesia, Huntington’s disease, and multiple sclerosis.

Our hypothesis was to detect TOS increase and TAS decrease in serum samples of patients with AD accompanied by insufficient levels of apelin-13. Thus, serum levels of apelin-13 might be useful as a screening test for AD.

While observing lower TAS in the blood of patients with ischemic stroke, Gariballa et al. reported an inverse correlation between plasma TAS and the extent of ischemia reperfusion-induced neurological damage. In a study by Yuan et al., patients with Parkinson’s disease were found to have significantly lower TAS, SOD, and Gpx (glutathione peroxidase) compared with healthy controls, with no apparent difference between groups in terms of plasma levels of vitamins A, C, and E. On the other hand, Sofic et al. did not observe any significant changes in plasma TAS of patients with AD, Parkinson’s disease, depression, or schizophrenia. Sinclair et al. reported on lower blood levels of vitamins C and E in patients with vascular dementia and AD compared with healthy controls, with no significant change in TAS.

In their study on patients with AD and older people individuals with mild cognitive impairment, Mecocci et al. managed to demonstrate a significant reduction in the levels of antioxidant enzymes, such as SOD and GPx, along with a decrease in serum levels of water-soluble and lipophilic antioxidants, namely vitamins A, C, E, and α-carotene. The authors also managed to establish the presence of an inverse correlation between plasma levels of several antioxidants, such as lycopene, lutein, α-carotene, and β-carotene, and lymphocytic DNA8-OHdG in patients with AD.

Minghetti et al. conducted a study in which 26 healthy controls with normal cognitive function were compared with 25 patients with AD (according to NINCDS-ADRDA diagnostic criteria) in terms of total antioxidant capacity in the serum. They showed that patients with AD had disrupted antioxidant defense systems compared with healthy controls, whereas also establishing a positive correlation between MMSE score and total reductive capacity. In our study, we also observed a statistically significant difference between the AD and control groups in terms of mean TAS measurements. Mean TAS in the AD group was lower than in the control group, and a statistically significant positive correlation was observed between MMSE scores and TAS values of patients showing the relation of cognitive functions indicated by MMSE scores with TAS/TOS status of patients with AD. These findings were consistent with the results of other studies by Mecocci et al. and Minghetti et al.

### 5 | CONCLUSION

In this study, we evaluated serum apelin-13 levels in patients with AD, a topic of research that was not studied much up to date. In addition, to the best of our knowledge, serum TOS has not been previously investigated in association with AD.
In this study, apelin-13 levels together with TAS values were lower in patients with AD with low MMSE scores in whom neurological damage seemed to occur. In addition, fair correlations among TOS, TAS, and apelin-13 levels as well as MMSE scores were observed. Our patients’ group consisted of newly diagnosed patients to prevent any pharmacological effect on biochemical pathways and serum levels of biomarkers. Monitorization of patients longer during the disease progression process may yield more significant relationships between these markers.

Studies with a large number of patients, including those with progressive AD, may provide more information about the diagnostic performance of apelin-13 as a biomarker for oxidative tissue damage. In case of contribution to clinical diagnosis, serum apelin-13 measurement may be easier, faster, and less invasive for patient care.

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**PRACTICE IMPACT STATEMENT**

Alzheimer’s disease, a progressive neurodegenerative disease, has also been linked to neuronal inflammation. Apelin-13, a predominant neuropeptide with inhibiting effect on inflammation, has beneficial effects on cognition memory and neuronal damage. In the following years, it may be possible to reduce the neuronal loss and memory loss of patients with treatments that will increase the amount of apelin-13 at an early stage.

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**CONFLICTS OF INTEREST**

Nothing to disclose.

**AUTHOR CONTRIBUTIONS**

Zeynep Yildiz: Conception of the design of the work, data acquisition, data analysis and interpretation, drafting the article, critically revising the article, final approval of the version to be published, and accountability for all aspects of the work. Nezaket Eren: Drafting the article, critically revising the article, and final approval of the version to be published. Asuman Orcun: Drafting the article, critically revising the article, and final approval of the version to be published. Fatma Munevver Gokyigit: Conception of the design of the work, data acquisition, data analysis and interpretation, drafting the article, critically revising the article, final approval of the version to be published, and accountability for all aspects of the work. Fatma Turgay: Conception of the design of the work, data acquisition, data analysis and interpretation, drafting the article, critically revising the article, final approval of the version to be published, and accountability for all aspects of the work. Lale Gundogdu Celebi: Conception of the design of the work, data acquisition, and data analysis and interpretation.

**TABLE 2** Result of correlation analyses between TAS, TOS, OSI, Apelin-13, and MMSE in the AD group

| Patient group | TOS (μmol H2O2 Eq/L) | TAS (Trolox Eq/L) | OSI (AU) | Apelin-13 (ng/mL) |
|---------------|----------------------|------------------|----------|------------------|
| TAS (Trolox Eq/L) r | -0.328 | | | |
| P value | 0.071 | | | |
| OSI (AU) r | 0.915 | -0.630 | | |
| P value | 0.001** | | | |
| Apelin-13 (ng/mL) r | -0.067 | 0.346 | -0.131 | |
| P value | 0.721 | 0.057 | 0.483 | |
| MMSE Score r | -0.444 | 0.368 | -0.529 | -0.305 |
| P value | 0.012* | 0.032* | 0.002** | 0.095 |

Note: r = Pearson’s correlation coefficient.
*P < 0.05; **P < 0.01.

Abbreviations: AD, Alzheimer’s disease; MMSE, Mini-Mental State Examination; OSI, Oxidative stress index; TAS, total antioxidant status; TOS, total oxidant status.
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